

Figure 1

GATCAAACCTCTTTTCCATTGAGAGTCCTCTGATTCAGATTTTAATGTTAACATTTTGGAAGACAGTATTCAGAAAAAAATTCC
TTAATAAAAAATACAACCTCAGATCCTTCAAATATGAACTGGTTGGGGAATCTCCATTTTTTCAATATTATTTTCTCTTTGTTTTC
TTGCTACGTATAATTATTAATATCCTGACTAGGTTGTGGTTGGAGGGTTATTACTTTTCATTTTACCATGCAGTCCAAATCTAAAC
TGCTTCTACTGATGGTTTACAGCATTCTGAGATAAGAATGGTACATCTAGAGAACATTTGCCAAAGGCCTAAGCACAGCAAAGGAA
AATAAACACAGAATATAATAAAATGAGATAATCTAGCTTAAACTATAACTTCCTCTTTAGAACTCCCAACCACATTTGGATC

Figure 1

FIG. 2A

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          9          18          27          36          45          54
5'  CAG AGA GGC TGT ATT TCA GTG CAG CCT GCC AGA CCT CTT CTG GAG GAA GAC TGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
          63          72          81          90          99          108
    ACA AAG GGG GTC ACA CAT TCC TTC CAT ACG GTT GAG CCT CTA CCT GCC TGG TGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

          117          126          135          144          153          162
    TGG TCA CAG TTC AGC TTC TTC ATG ATG GTG GAT CCC AAT GGC AAT GAA TCC AGT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                M  M  V  D  P  N  G  N  E  S  S

          171          180          189          198          207          216
    GCT ACA TAC TTC ATC CTA ATA GGC CTC CCT GGT TTA GAA GAG GCT CAG TTC TGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    A  T  Y  F  I  L  I  G  L  P  G  L  E  E  A  Q  F  W

          225          234          243          252          261          270
    TTG GCC TTC CCA TTG TGC TCC CTC TAC CTT ATT GCT GTG CTA GGT AAC TTG ACA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    L  A  F  P  L  C  S  L  Y  L  I  A  V  L  G  N  L  T

          279          288          297          306          315          324
    ATC ATC TAC ATT GTG CGG ACT GAG CAC AGC CTG CAT GAG CCC ATG TAT ATA TTT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    I  I  Y  I  V  R  T  E  H  S  L  H  E  P  M  Y  I  F

          333          342          351          360          369          378
    CTT TGC ATG CTT TCA GGC ATT GAC ATC CTC ATC TCC ACC TCA TCC ATG CCC AAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    L  C  M  L  S  G  I  D  I  L  I  S  T  S  S  M  P  K

          387          396          405          414          423          432
    ATG CTG GCC ATC TTC TGG TTC AAT TCC ACT ACC ATC CAG TTT GAT GCT TGT CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    M  L  A  I  F  W  F  N  S  T  T  I  Q  F  D  A  C  L

          441          450          459          468          477          486
    CTA CAG ATT TTT GCC ATC CAC TCC TTA TCT GGC ATG GAA TCC ACA GTG CTG CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    L  Q  I  F  A  I  H  S  L  S  G  M  E  S  T  V  L  L

          495          504          513          522          531          540
    GCC ATG GCT TTT GAC CGC TAT GTG GCC ATC TGT CAC CCA CTG CGC CAT GCC ACA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    A  M  A  F  D  R  Y  V  A  I  C  H  P  L  R  H  A  T

          549          558          567          576          585          594
    GTA CTT ACG TTG CCT CGT GTC ACC AAA ATT GGT GTG GCT GCT GTG GTG CGG GGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    V  L  T  L  P  R  V  T  K  I  G  V  A  A  V  V  R  G

          603          612          621          630          639          648
    GCT GCA CTG ATG GCA CCC CTT CCT GTC TTC ATC AAG CAG CTG CCC TTC TGC CGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    A  A  L  M  A  P  L  P  V  F  I  K  Q  L  P  F  C  R

```

FIG. 2B

657	666	675	684	693	702
TCC AAT ATC CTT TCC CAT TCC TAC TGC CTA CAC CAA GAT GTC ATG AAG CTG GCC					
S N I L S H S Y C L H Q D V M K L A					
711	720	729	738	747	756
TGT GAT GAT ATC CGG GTC AAT GTC GTC TAT GGC CTT ATC GTC ATC ATC TCC GCC					
C D D I R V N V V Y G L I V I I S A					
765	774	783	792	801	810
ATT GGC CTG GAC TCA CTT CTC ATC TCC TTC TCA TAT CTG CTT ATT CTT AAG ACT					
I G L D S L L I S F S Y L L I L K T					
819	828	837	846	855	864
GTG TTG GGC TTG ACA CGT GAA GCC CAG GCC AAG GCA TTT GGC ACT TGC GTC TCT					
V L G L T R E A Q A K A F G T C V S					
873	882	891	900	909	918
CAT GTG TGT GCT GTG TTC ATA TTC TAT GTA CCT TTC ATT GGA TTG TCC ATG GTG					
H V C A V F I F Y V P F I G L S M V					
927	936	945	954	963	972
CAT CGC TTT AGC AAG CGG CGT GAC TCT CCG CTG CCC GTC ATC TTG GCC AAT ATC					
H R F S K R R D S P L P V I L A N I					
981	990	999	1008	1017	1026
TAT CTG CTG GTT CCT CCT GTG CTC AAC CCA ATT GTC TAT GGA GTG AAG ACA AAG					
Y L L V P P V L N P I V Y G V K T K					
1035	1044	1053	1062	1071	1080
GAG ATT CGA CAG CGC ATC CTT CGA CTT TTC CAT GTG GCC ACA CAC GCT TCA GAG					
E I R Q R I L R L F H V A T H A S E					
1089	1098	1107	1116	1125	1134
CCC TAG GTG TCA GTG ATC AAA CTT CTT TTC CAT TCA GAG TCC TCT GAT TCA GAT					
P *					
1143	1152	1161	1170	1179	1188
TTT AAT GTT AAC ATT TTG GAA GAC AGT ATT CAG AAA AAA AAT TTC CTT AAT AAA					
1197	1206	1215	1224	1233	1242
AAA TAC AAC TCA GAT CCT TCA AAT ATG AAA CTG GTT GGG GAA TCT CCA TTT TTT					
1251	1260	1269	1278	1287	1296
CAA TAT TAT TTT CTT CTT TGT TTT CTT GCT ACA TAT AAT TAT TAA TAC CCT GAC					
1305	1314	1323	1332	1341	1350
TAG GTT GTG GTT GGA GGG TTA TTA CTT TTC ATT TTA CCA TGC AGT CCA AAT CTA					

FIG. 2C

1359	1368	1377	1386	1395	1404
AAC TGC TTC	TAC TGA TGG	TTT ACA GCA	TTC TGA GAT	AAG AAT GGT	ACA TCT AGA
1413	1422	1431	1440	1449	1458
GAA CAT TTG	CCA AAG GCC	TAA GCA CGG	CAA AGG AAA	ATA AAC ACA	GAA TAT AAT
1467	1476	1485	1494	1503	1512
AAA ATG AGA	TAA TCT AGC	TTA AAA CTA	TAA CTT CCT	CTT CAG AAC	TCC CAA CCA
1521	1530	1539	1548	1557	1566
CAT TGG ATC	TCA GAA AAA	TGC TGT CTT	CAA AAT GAC	TTC TAC AGA	GAA GAA ATA
1575	1584	1593	1602	1611	1620
ATT TTT CCT	CTG GAC ACT	AGC ACT TAA	GGG GAA GAT	TGG AAG TAA	AGC CTT GAA
1629	1638	1647	1656	1665	1674
AAG AGT ACA	TTT ACC TAC	GTT AAT GAA	AGT TGA CAC	ACT GTT CTG	AGA GTT TTC
1683	1692	1701	1710	1719	1728
ACA GCA TAT	GGA CCC TGT	TTT TCC TAT	TTA ATT TTC	TTA TCA ACC	CTT TAA TTA
1737	1746	1755	1764	1773	1782
GGC AAA GAT	ATT ATT AGT	ACC CTC ATT	GTA GCC ATG	GGA AAA TTG	ATG TTC AGT
1791	1800	1809	1818	1827	1836
GGG GAT CAG	TGA ATT AAA	TGG GGT CAT	ACA AGT ATA	AAA ATT AAA	AAA AAA AAA
1845	1854	1863	1872	1881	1890
GAC TTC ATG	CCC AAT CTC	ATA TGA TGT	GGA AGA ACT	GTT AGA GAG	ACC AAC AGG
1899	1908	1917	1926	1935	1944
GTA GTG GGT	TAG AGA TTT	CCA GAG TCT	TAC ATT TTC	TAG AGG AGG	TAT TTA ATT
1953	1962	1971	1980	1989	1998
TCT TCT CAC	TCA TCC AGT	GTT GTA TTT	AGG AAT TTC	CTG GCA ACA	GAA CTC ATG
2007	2016	2025	2034	2043	2052
GCT TTA ATC	CCA CTA GCT	ATT GCT TAT	TGT CCT GGT	CCA ATT GCC	AAT TAC CTG
2061	2070	2079	2088	2097	2106
TGT CTT GGA	AGA AGT GAT	TTC TAG GTT	CAC CAT TAT	GGA AGA TTC	TTA TTC AGA
2115	2124	2133	2142	2151	2160
AAG TCT GCA	TAG GGC TTA	TAG CAA GTT	ATT TAT TTT	TAA AAG TTC	CAT AGG TGA
2169	2178	2187	2196	2205	2214
TTC TGA TAG	GCA GTG AGG	TTA GGG AGC	CAC CAG TTA	TGA TGG GAA	GTA TGG AAT
2223	2232	2241	2250	2259	2268
GGC AGG TCT	TGA AGA TAA	CAT TGG CCT	TTT GAG TGT	GAC TCG TAG	CTG GAA AGT
2277	2286	2295	2304	2313	2322
GAG GGA ATC	TTC AGG ACC	ATG CTT TAT	TTG GGG CTT	TGT GCA GTA	TGG AAC AGG
2331	2340	2349	2358	2367	2376
GAC TTT GAG	ACC AGG AAA	GCA ATC TGA	CTT AGG CAT	GGG AAT CAG	GCA TTT TTG

FIG. 2D

2385	2394	2403	2412	2421	2430
CTT CTG AGG GGC TAT TAC CAA GGG TTA ATA GGT TTC ATC TTC AAC AGG ATA TGA					
2439	2448	2457	2466	2475	2484
CAA CAG TGT TAA CCA AGA AAC TCA AAT TAC AAA TAC TAA AAC ATG TGA TCA TAT					
2493	2502	2511	2520	2529	2538
ATG TGG TAA GTT TCA TTT TCT TTT TCA ATC CTC AGG TTC CCT GAT ATG GAT TCC					
2547	2556	2565	2574	2583	2592
TAT AAC ATG CTT TCA TCC CCT TTT GTA ATG GAT ATC ATA TTT GGA AAT GCC TAT					
2601	2610	2619	2628	2637	2646
TTA ATA CTT GTA TTT GCT GCT GGA CTG TAA GCC CAT GAG GGC ACT GTT TAT TAT					
2655	2664	2673	2682	2691	2700
TGA ATG TCA TCT CTG TTC ATC ATT GAC TGC TCT TTG CTC ATC ATT GAA TCC CCC					
2709	2718	2727	2736	2745	2754
AGC AAA GTG CCT AGA ACA TAA TAG TGC TTA TGC TTG ACA CCG GTT ATT TTT CAT					
2763	2772	2781	2790	2799	2808
CAA ACC TGA TTC CTT CTG TCC TGA ACA CAT AGC CAG GCA ATT TTC CAG CCT TCT					
2817	2826	2835	2844	2853	2862
TTG AGT TGG GTA TTA TTA AAT TCT GGC CAT TAC TTC CAA TGT GAG TGG AAG TGA					
2871	2880	2889	2898	2907	2916
CAT GTG CAA TTT CTA TAC CTG GCT CAT AAA ACC CTC CCA TGT GCA GCC TTT CAT					
2925	2934	2943	2952	2961	2970
GTT GAC ATT AAA TGT GAC TTG GGA AGC TAT GTG TTA CAC AGA GTA AAT CAC CAG					
2979	2988	2997	3006	3015	3024
AAG CCT GGA TTT CTG AAA AAA CTG TGC AGA GCC AAA CCT CTG TCA TTT GCA ACT					
3033	3042	3051	3060	3069	3078
CCC ACT TGT ATT TGT ACG AGG CAG TTG GAT AAG TGA AAA ATA AAG TAC TAT TGT					
3087	3096	3105	3114	3123	3132
GTC AAG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA					

AAA A 3'

Figure 3: Protein Sequence for 101P3A11.

MVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDILI
STSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGV
AAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVVYGLIVIISAIGLDSLLISFSYL
LILKTVLGLTREAQAKAFGTCVSHVCAVFIFYVPFIGLSMVHRFSKRSDPLPVILANIYLLVPPVLNPIVYG
VKTKEIRQRILRLFHVATHASEP

sd-61572

Figure 4

Alignment of 101P3A11 (Sbjct) with mouse olfactory receptor S25 (Query)

Query: 34 GNYTVVTEFILLGLTDDITVSVILFVMFLIVYSVTLMGNLNIIVLIRTSPQLHTPMYLFL 93
GN + T FIL+GL L +Y + ++GNL II ++RT LH PMY+FL
Sbjct: 6 GNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFL 65

Query: 94 SHLAFLDIGYSSSVTPIMLRGFLRKGTFFPVAGCVAQLCIVVAFGTSESFLLASMAYDRY 153
L+ +DI S+S P ML F T I C+ Q+ + + ES +L +MA+DRY
Sbjct: 66 CMLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRY 125

Query: 154 VAICSPLLYSTQMSSTVCILLVGTSYLGWVNAWIFTGCSLNL SFCGPNKINHFFCDYSP 213
VAIC PL ++T ++ + + + G L FC N ++H +C +
Sbjct: 126 VAICHPLRHATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQD 185

Query: 214 LLKLSCSHDFSFEVIPAISGSIIVVTVFIIALSIVYILVSILKMRSTEGRQKAFSTCTS 273
++KL+C V I S I + +I+ SY+ IL ++L + + E + KAF TC S
Sbjct: 186 VMKLACDDIRVNVVYGLIIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVS 244

Query: 274 HLTAVTLFFGTITFIYVMPQSSYSTQNK---VVSVFYTVVIPMLNPLIYSFRNKEVKE 329
H+ AV +F+ + FI + +S ++ +++ Y +V P+LNP++Y + KE+++
Sbjct: 245 HVCAVFIFY--VPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLNPIVYGVKTKAIRQ 302

Query: 330 AMKKL 334
+ +L
Sbjct: 303 RILRL 307

Figure 5:

101P3A11 Hydrophilicity profile

(Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)

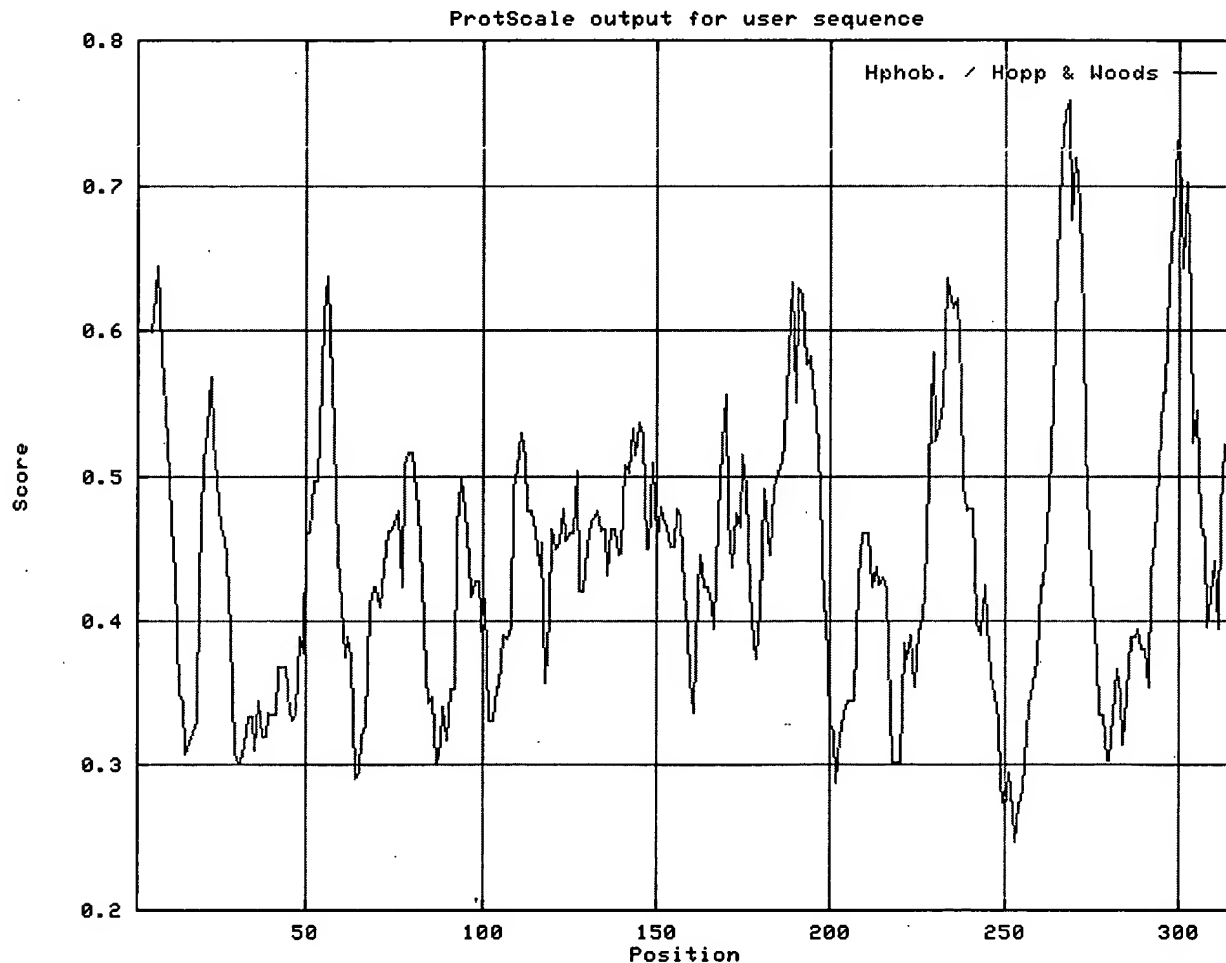


Figure 6:
101P3A11 Hydropathicity Profile
(Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)

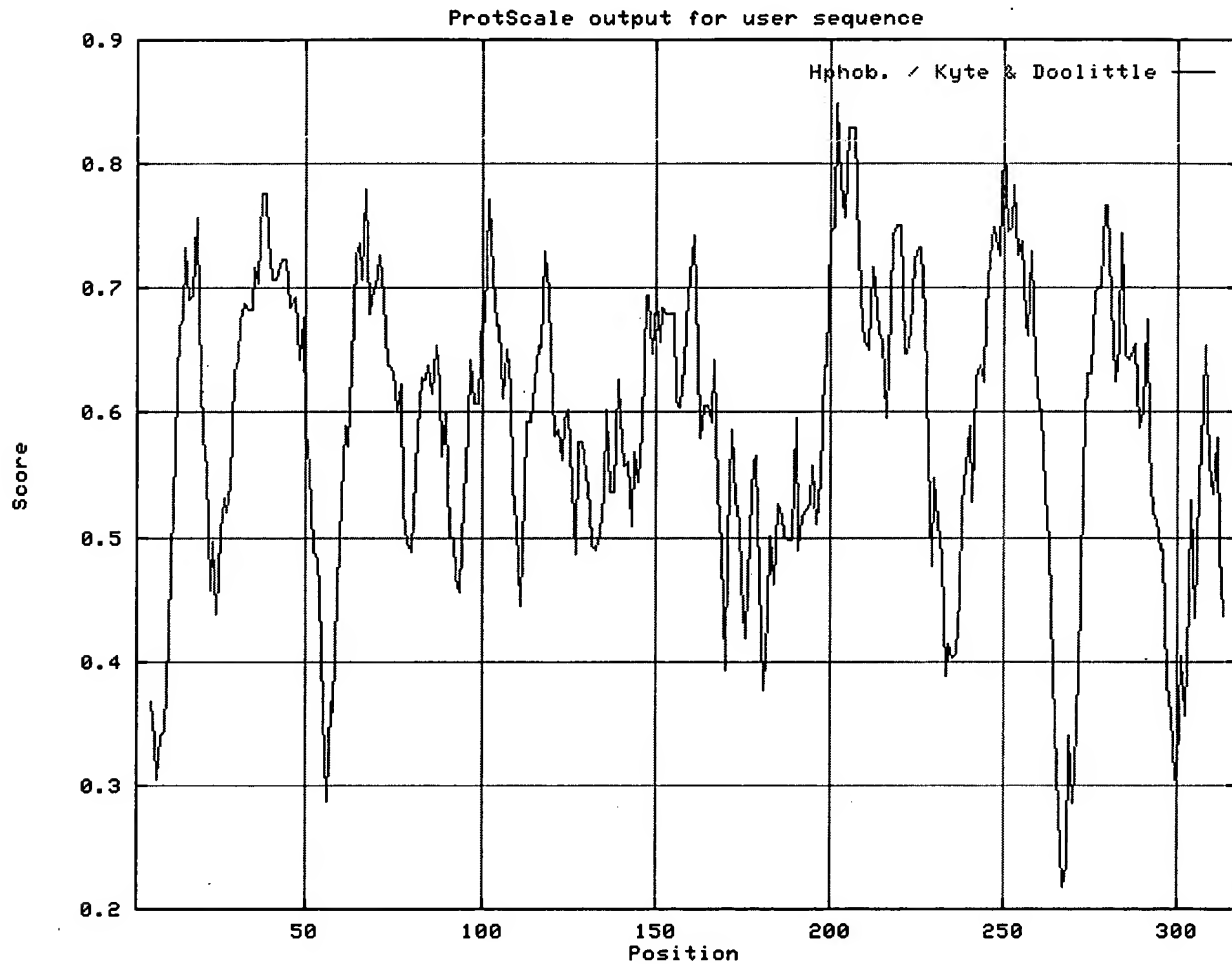


Figure 7:
101P3A11 % Accessible Residues Profile
(Janin J., 1979. Nature 277:491-492)

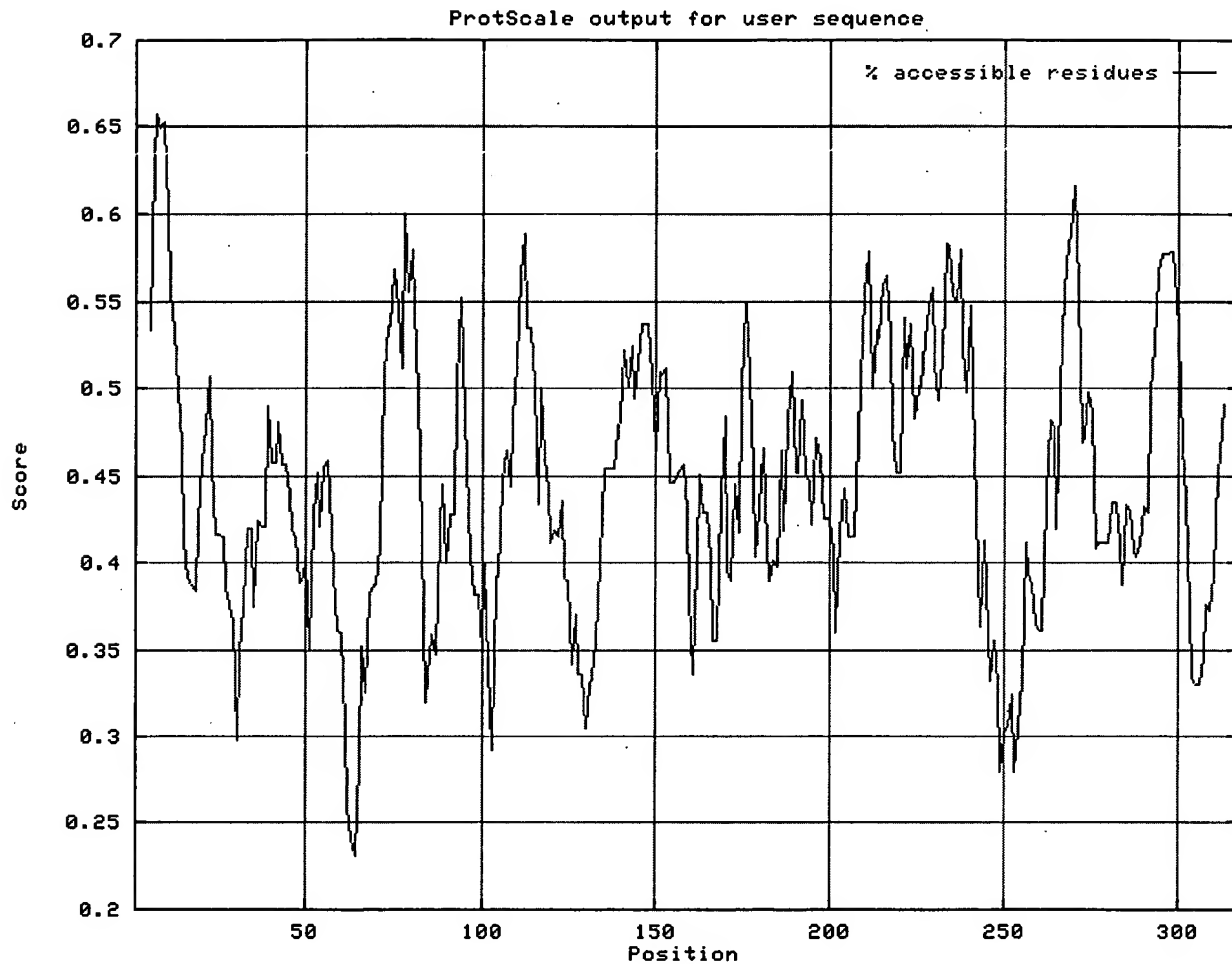


Figure 8:
101P3A11 Average Flexibility Profile
(Bhaskaran R., Ponnuswamy P.K., 1988.
Int. J. Pept. Protein Res. 32:242-255)

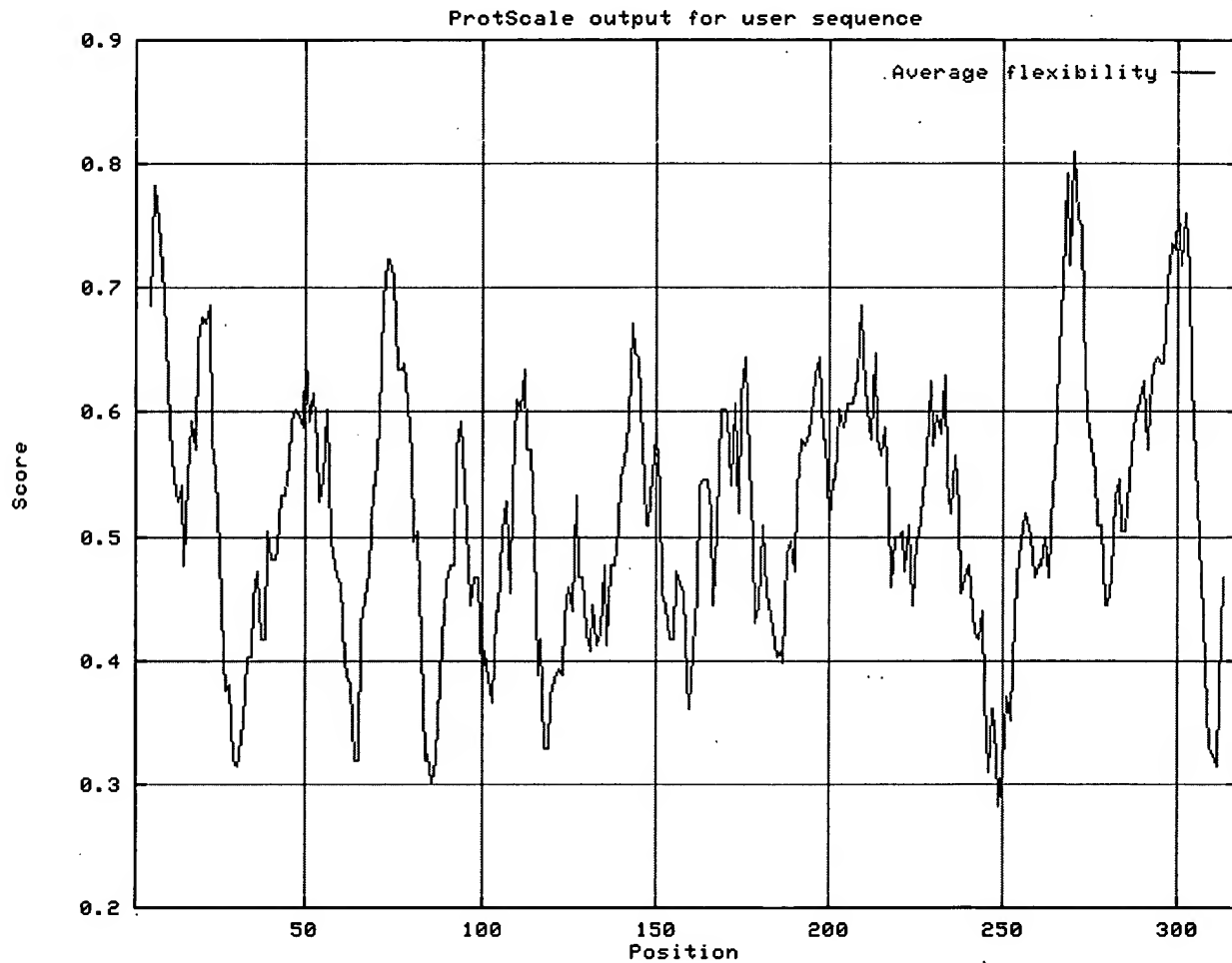


Figure 9:
101P3A11 Beta-turn Profile
(Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)

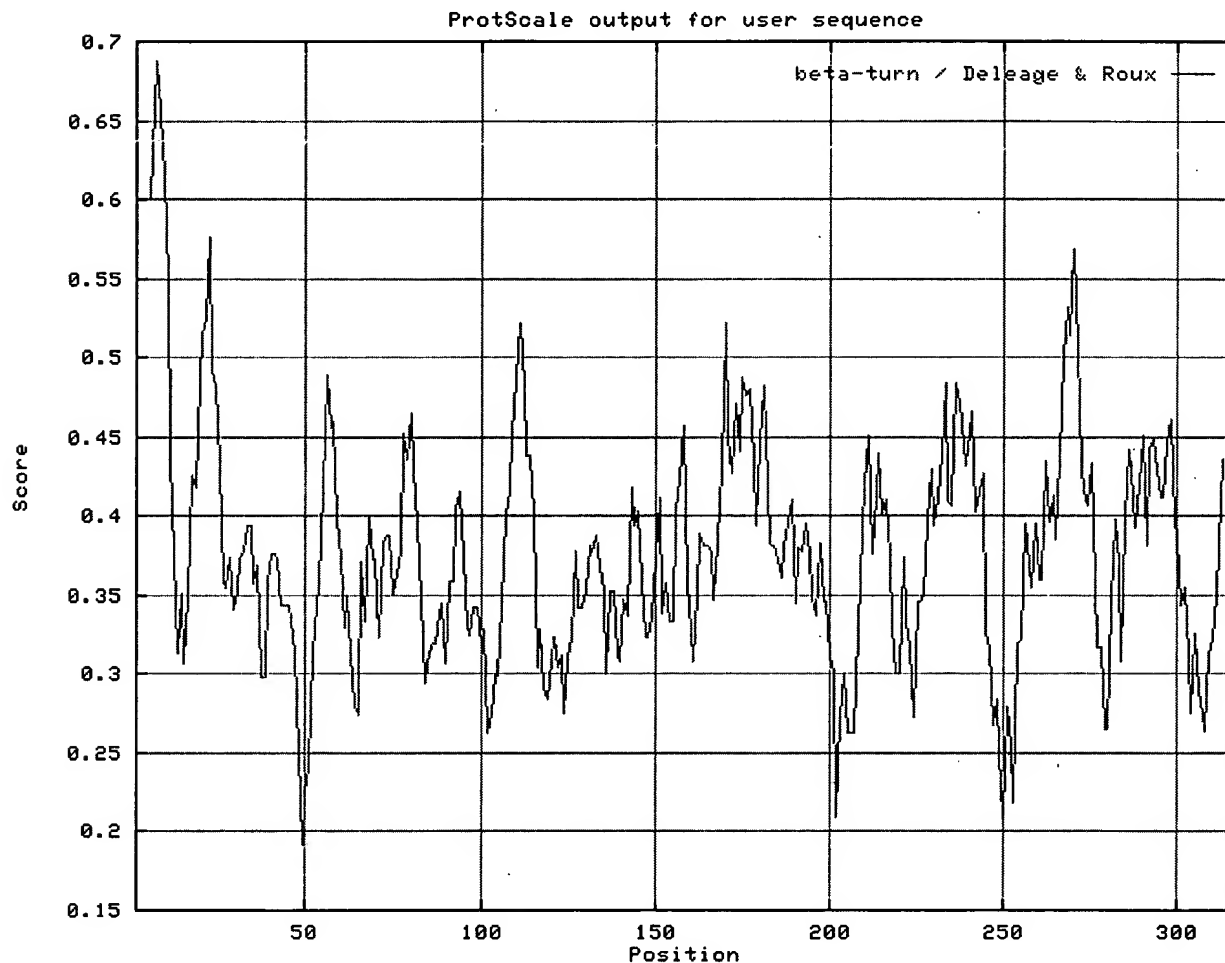


FIGURE 10A

Figure 10A. Expression of 101P3A11 by RT-PCR

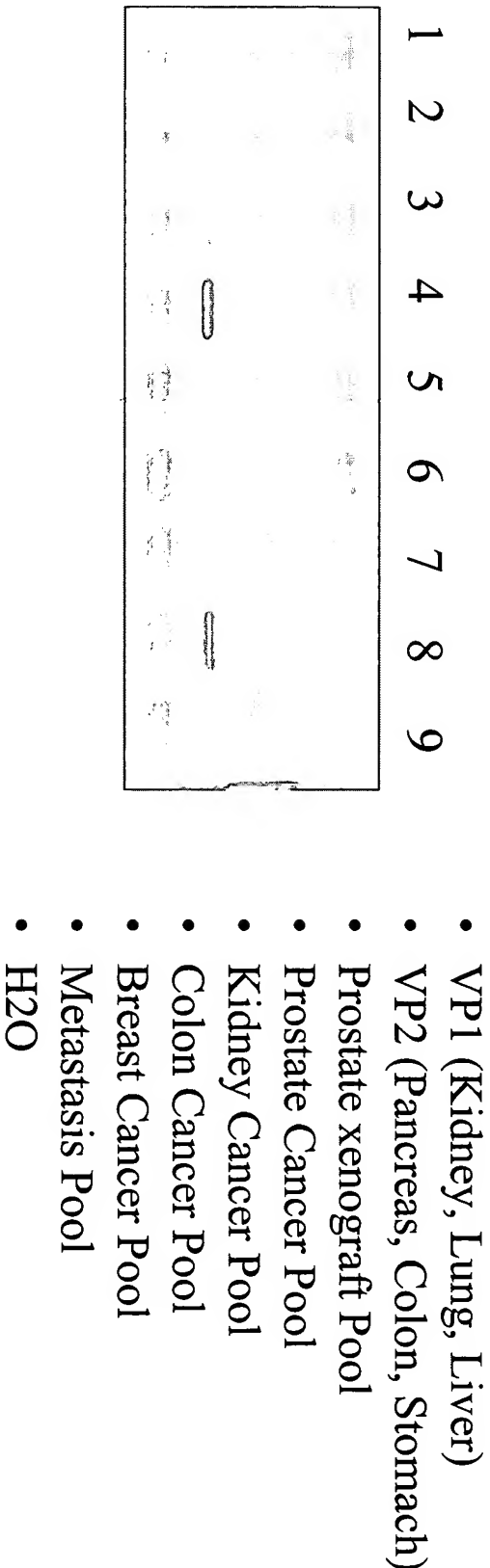


FIG. 10B

Figure 10B

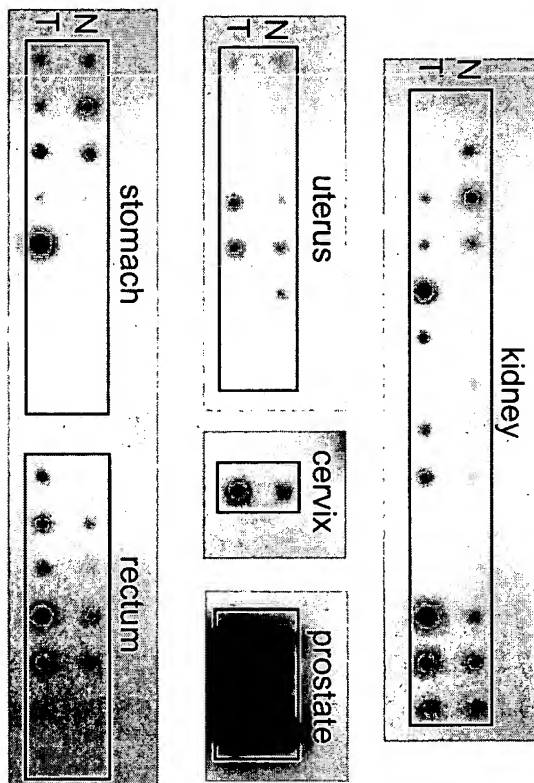
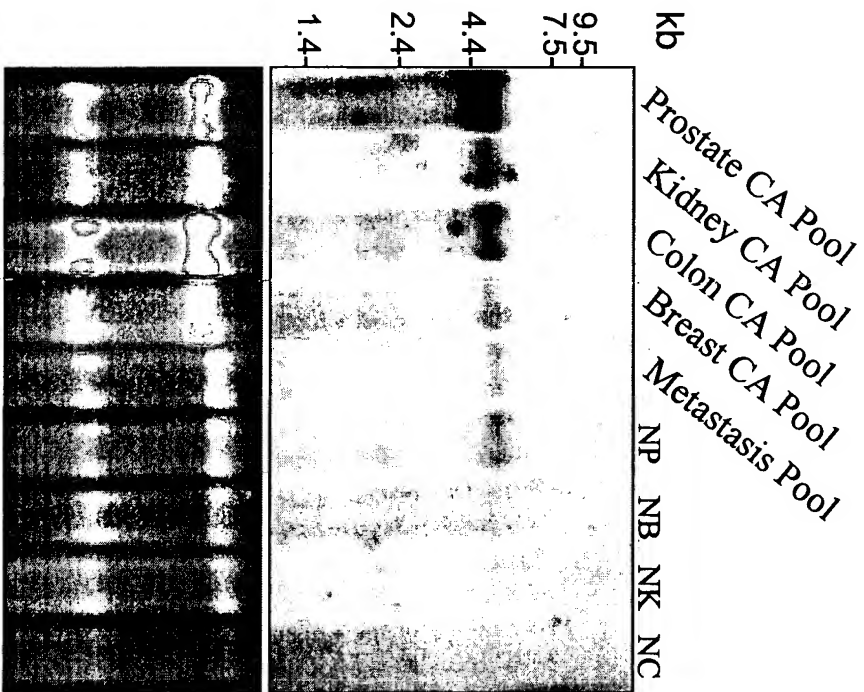


Figure 11. Expression of 101P3A11 in Human Patient Cancer Specimens



10µg total RNA/per lane from a pool of 3 tumors as follows:

Prostate Cancer Pool = gleason 6, 8, 9

Kidney Cancer Pool = grade 2, 2, 3

Colon Cancer Pool = stage II, III, IV

Breast Cancer Pool = grade 1, 2, 3

Metastasis Pool = colon to lung, colon to liver, ovary to fall. tube

NP = Normal Prostate

NB = Normal Bladder

NK = Normal Kidney

NC = Normal Colon

Figure 12A

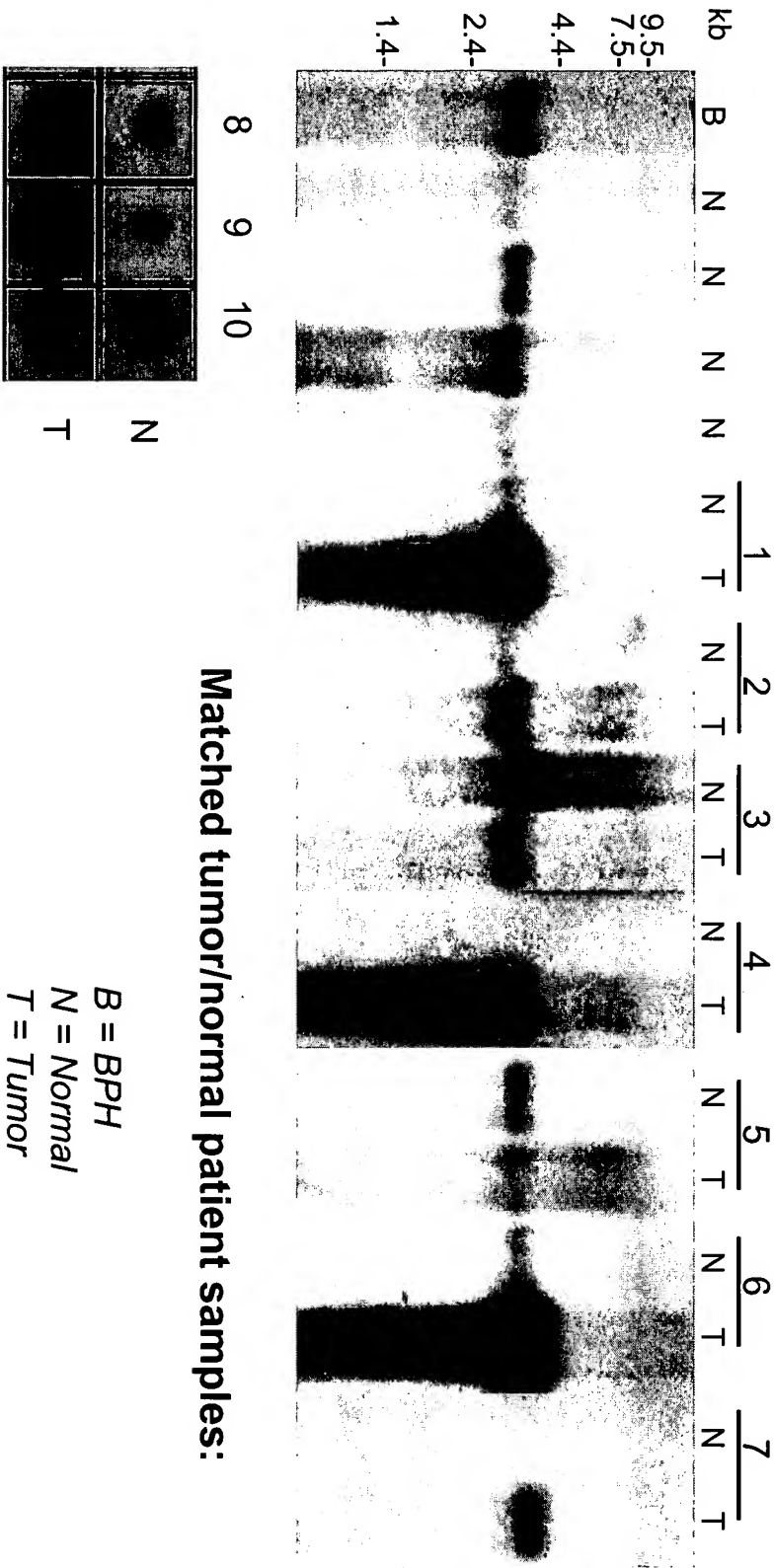


Figure 12B and 12C

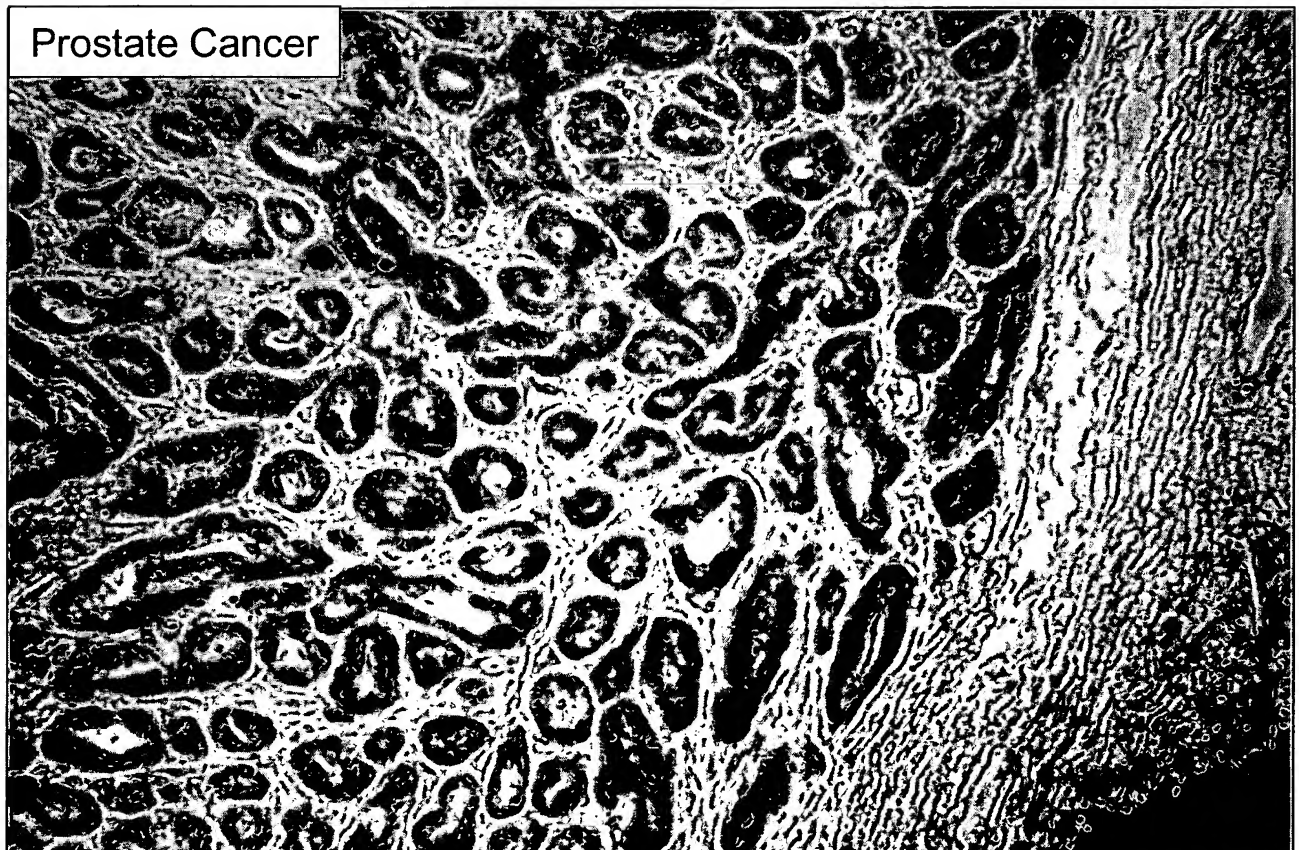
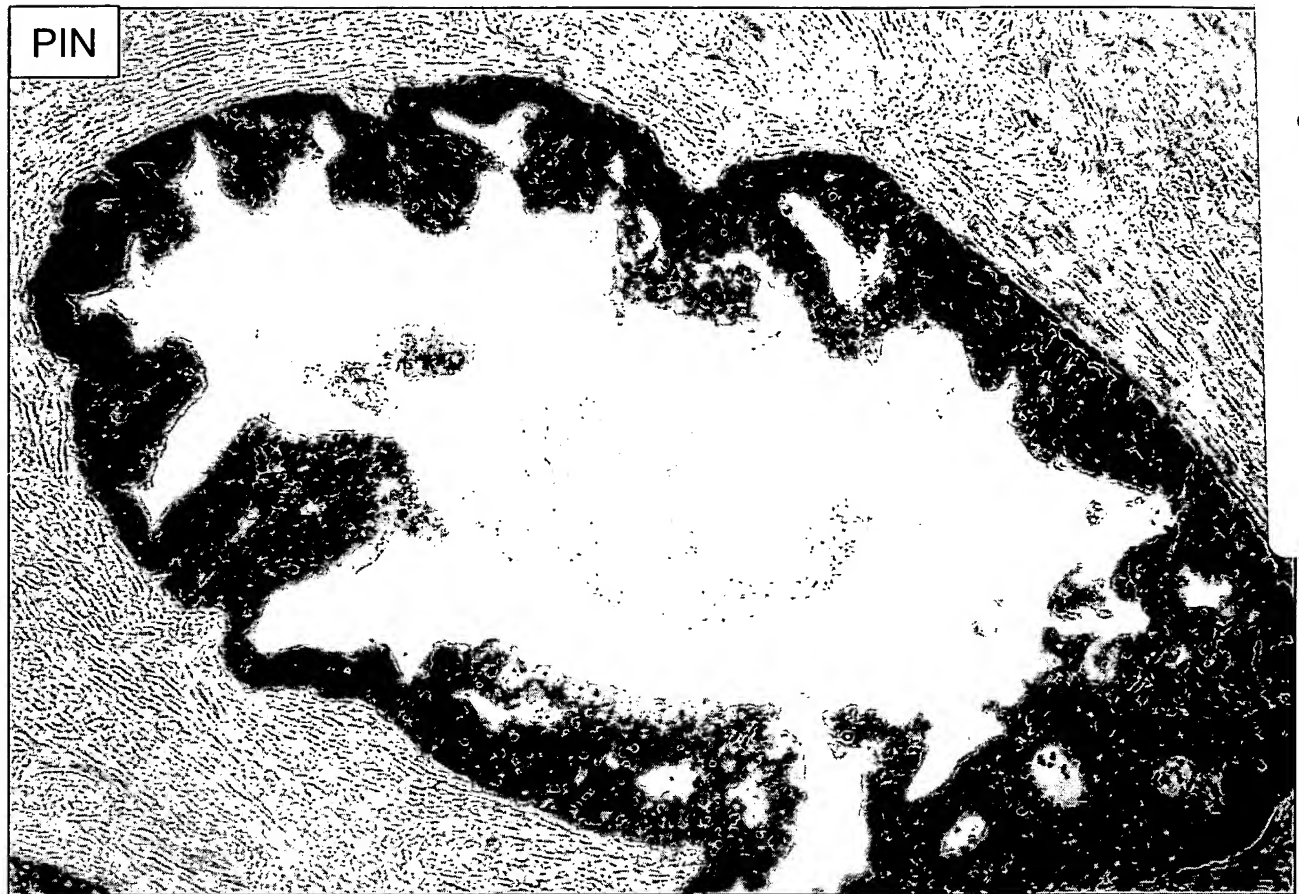
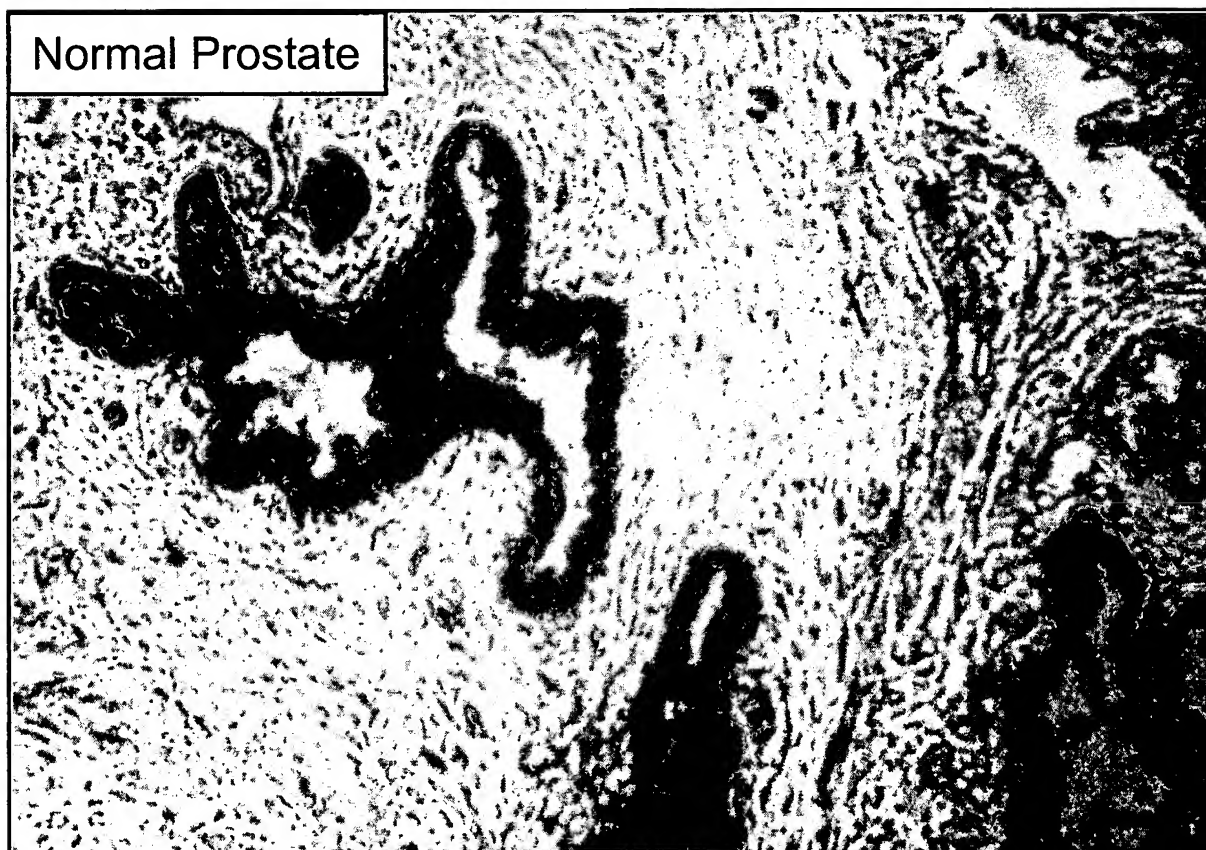
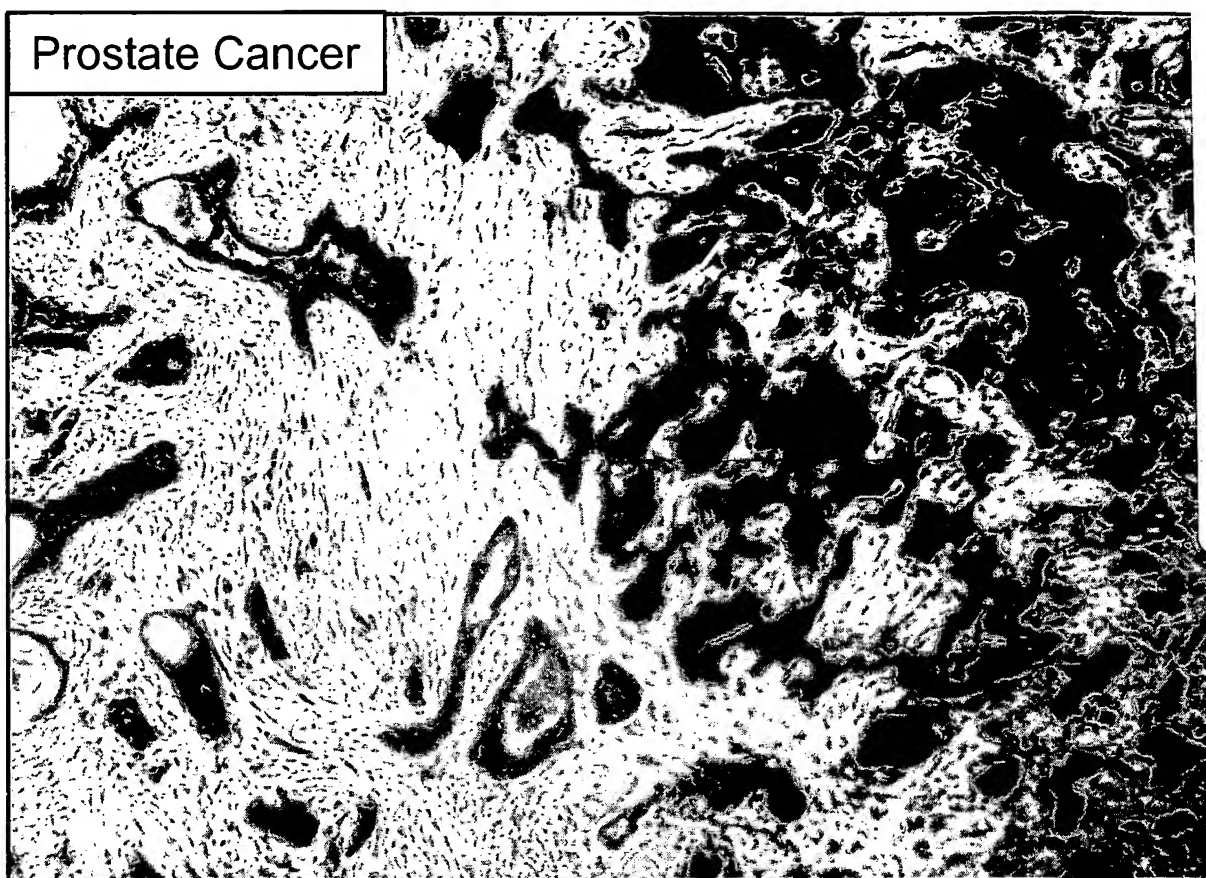
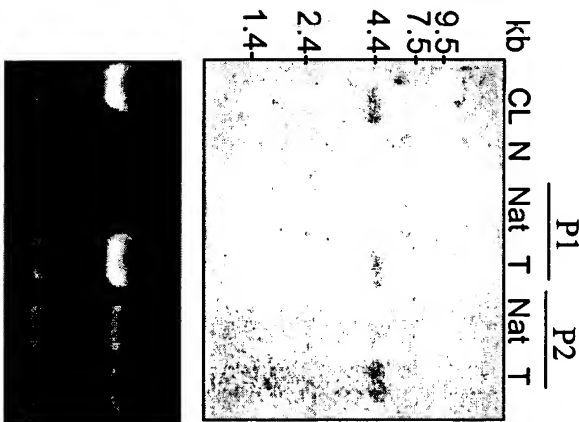


Figure 12D and 12E



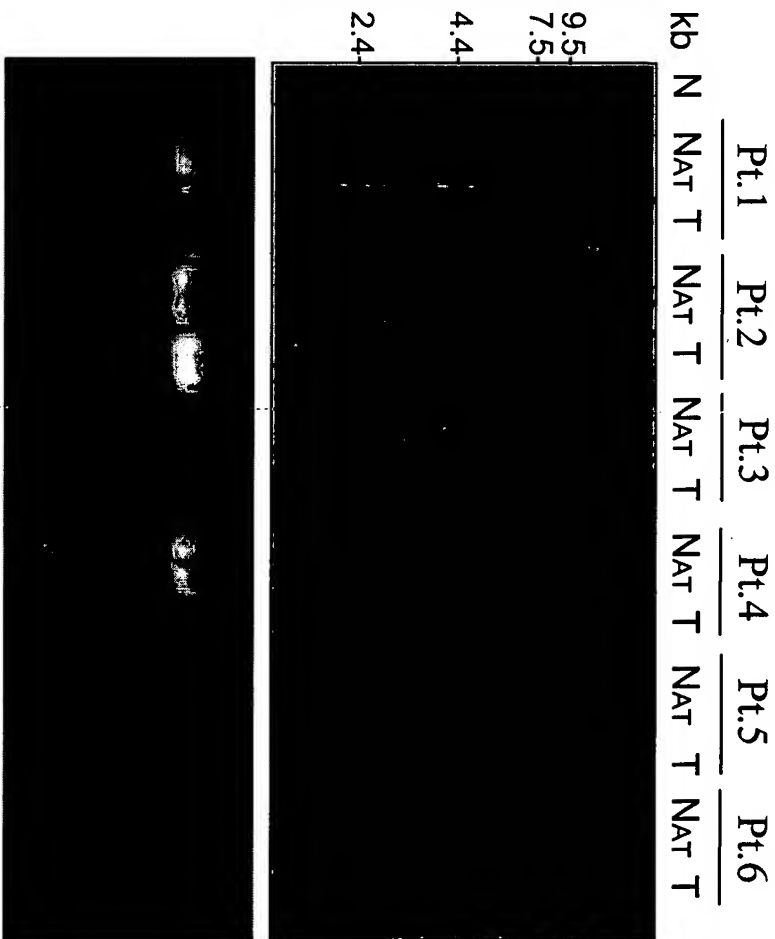
10001459-1000101

Figure 13. Expression of 101P3A11 in Colon Cancer Patient Specimens



P1, Stage I
P2, Stage IV
CL = Colon cancer cell line T84
N = Normal colon
Nat = Normal Adjacent Tissue
T = Tumor

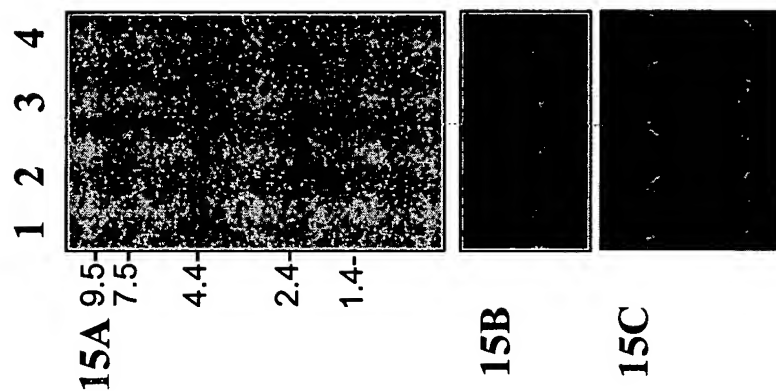
Figure 14. Expression of 101P3A11 in Kidney Cancer Patient Specimens



Pt.1, Papillary carcinoma, stage I
Pt.2, Invasive papillary carcinoma
Pt.3, Clear cell type grade 1/3, focally 2/3
Pt.4, Clear cell type, stage III
Pt.5, Clear cell type, stage III
Pt.6, Clear cell type, stage III

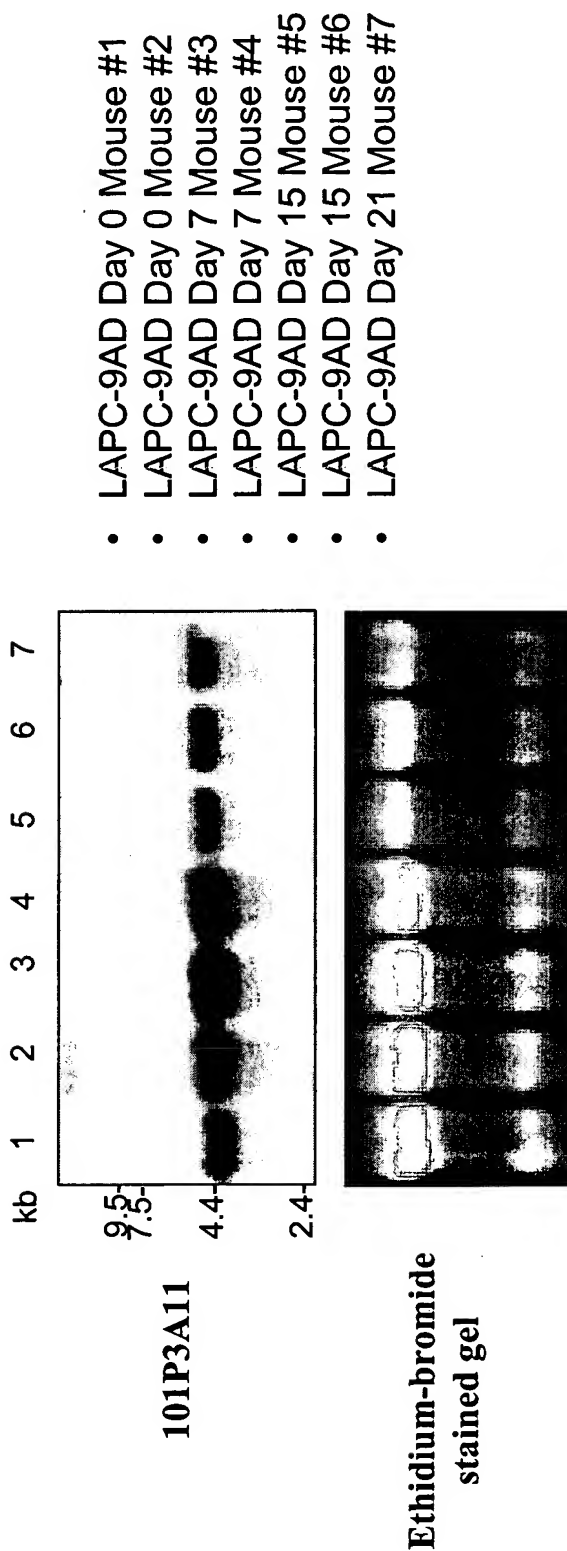
N = Normal kidney
Nat = Normal adjacent tumor
T = Tumor

Figure 15A-15C. Androgen Regulation of 101P3A11 in Tissue Culture Cells

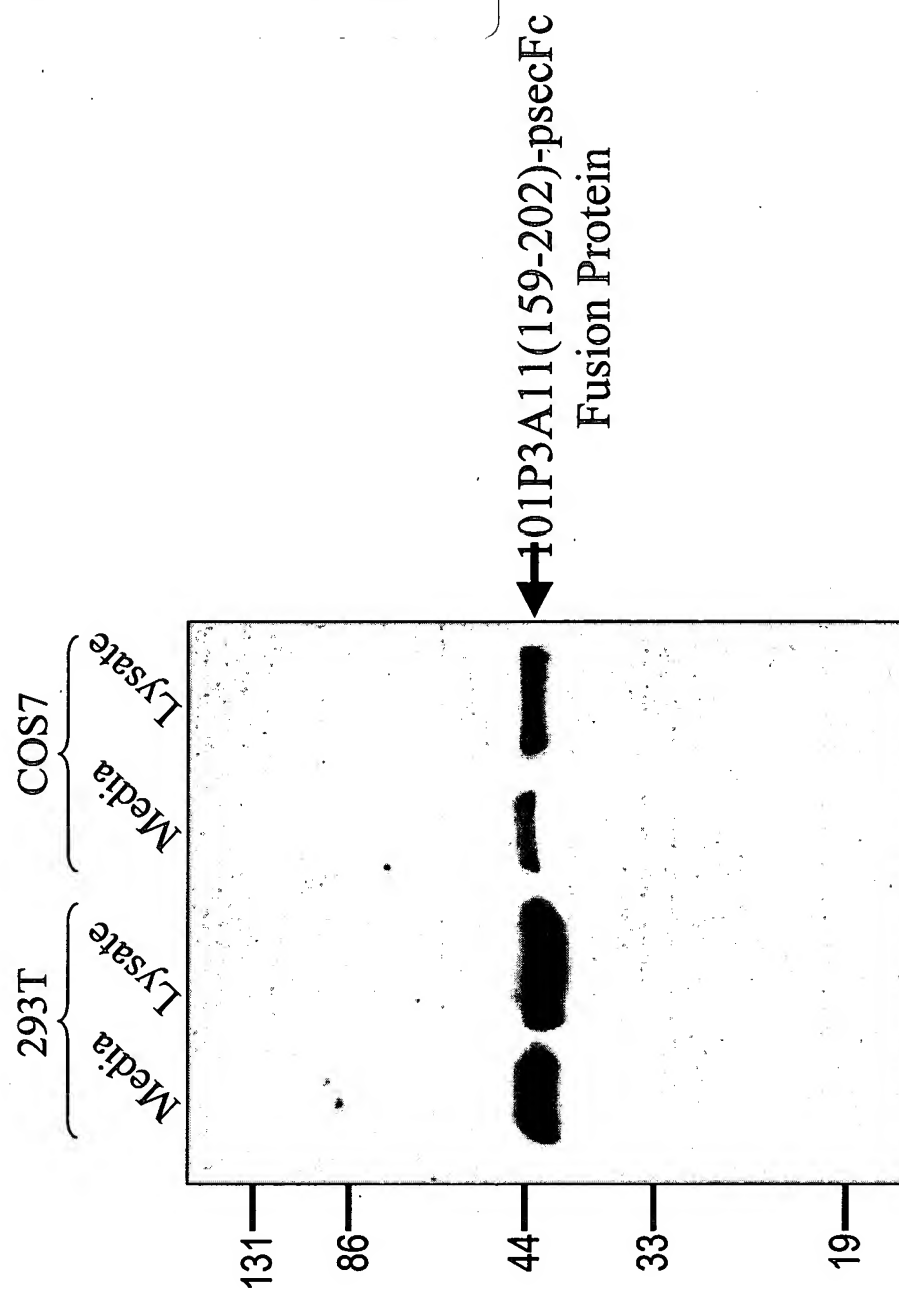


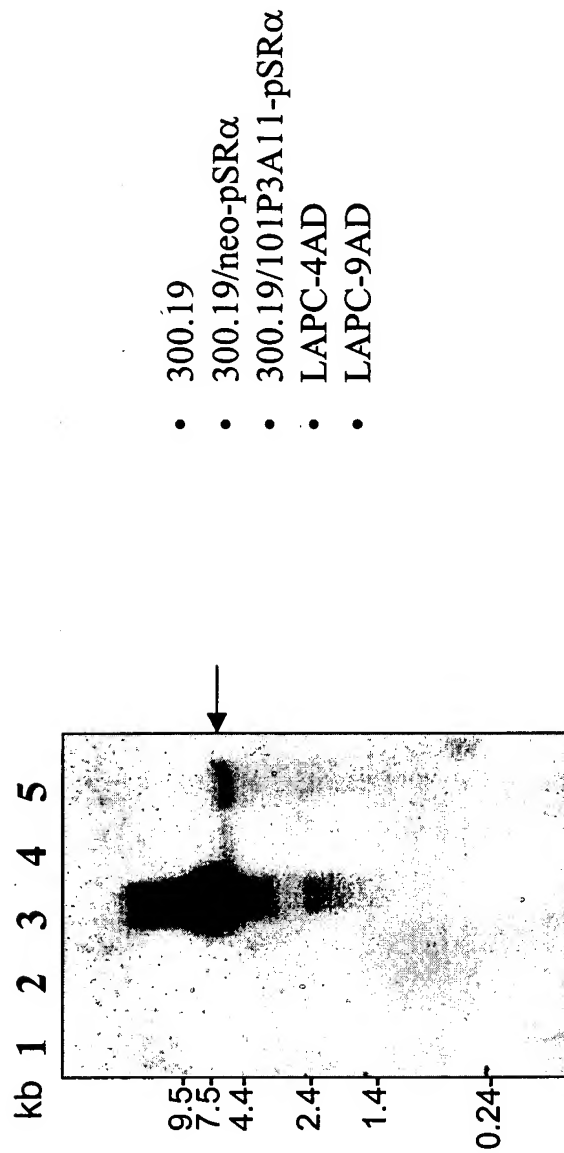
- LAPC-9 cell line charcoal-stripped FBS, 14 hrs
- LAPC-9 cell line charcoal-stripped FBS, 14 hrs + mibolerone
- LAPC-9 cell line charcoal-stripped FBS, 24 hrs
- LAPC-9 cell line charcoal-stripped FBS, 24 hrs + mibolerone

Figure 16. Androgen Regulation of 101P3A11 *In Vivo*



**Figure 17. Expression and Detection of 101P3A11(159-202)-
psecFc Fusion Protein**



[illegible]

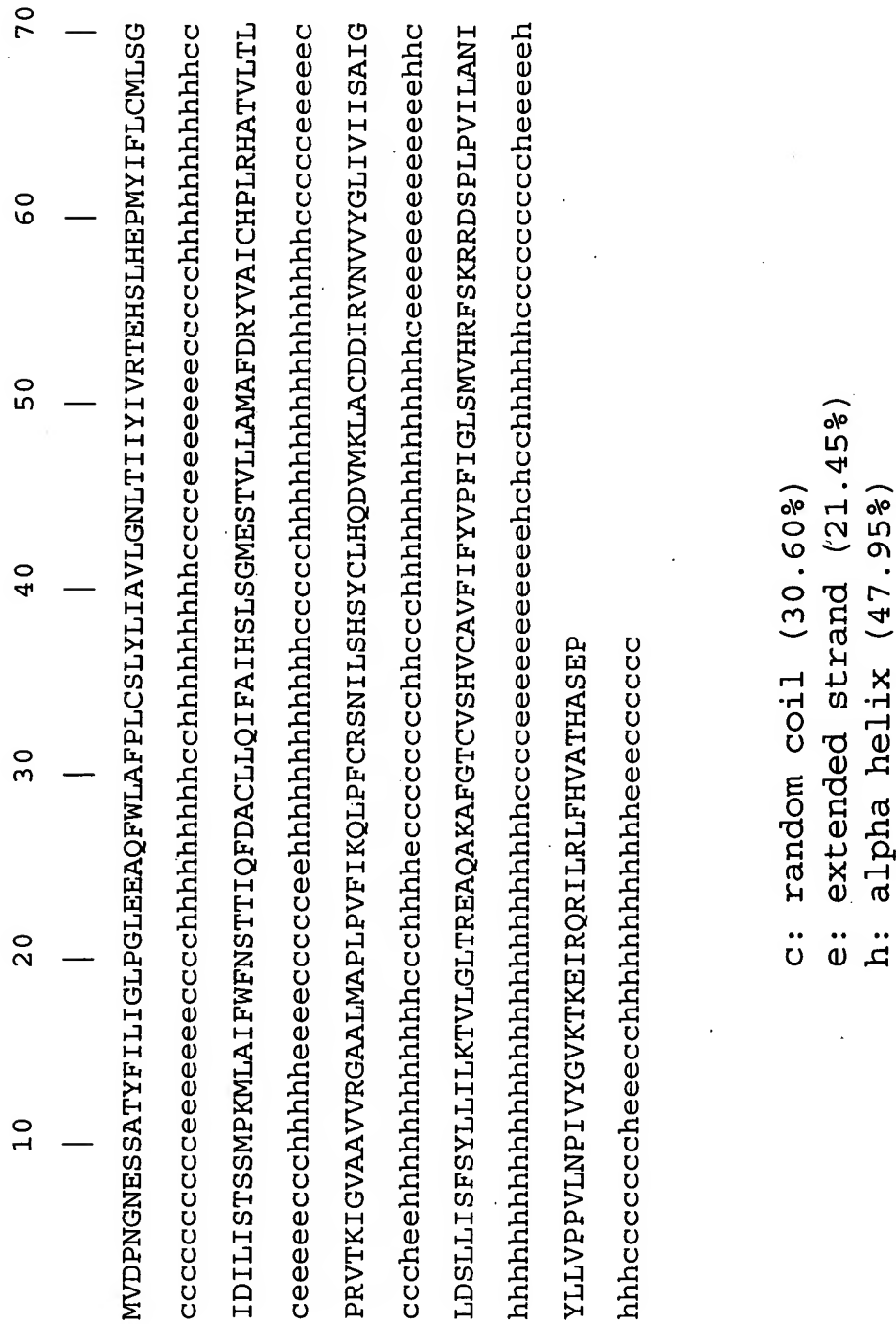
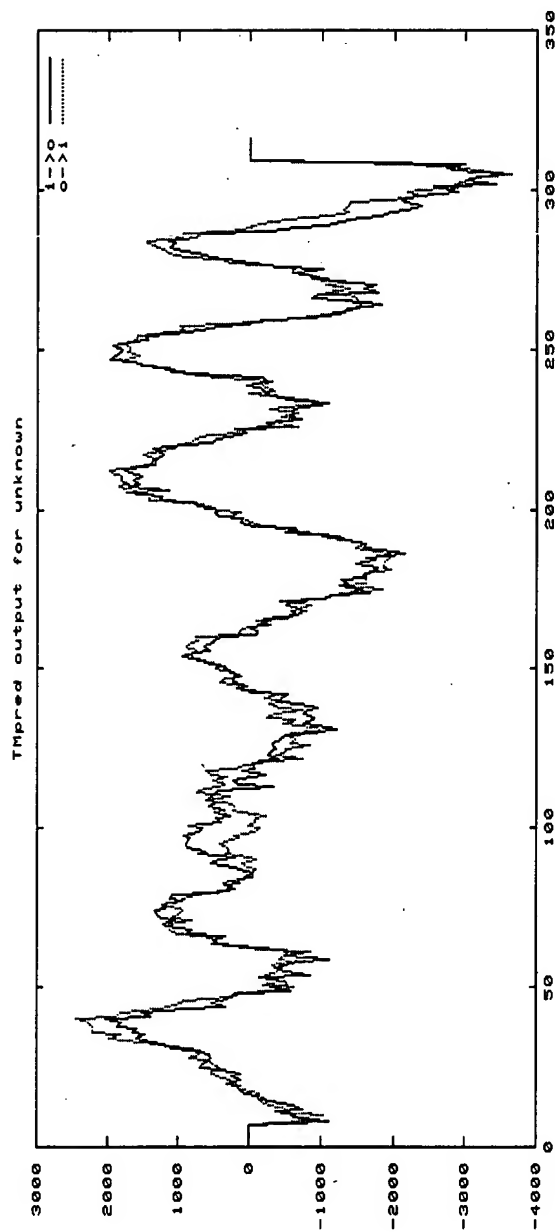
[illegible]

Figure 19B-19C. Transmembrane prediction of 101P3A11

19B



19C

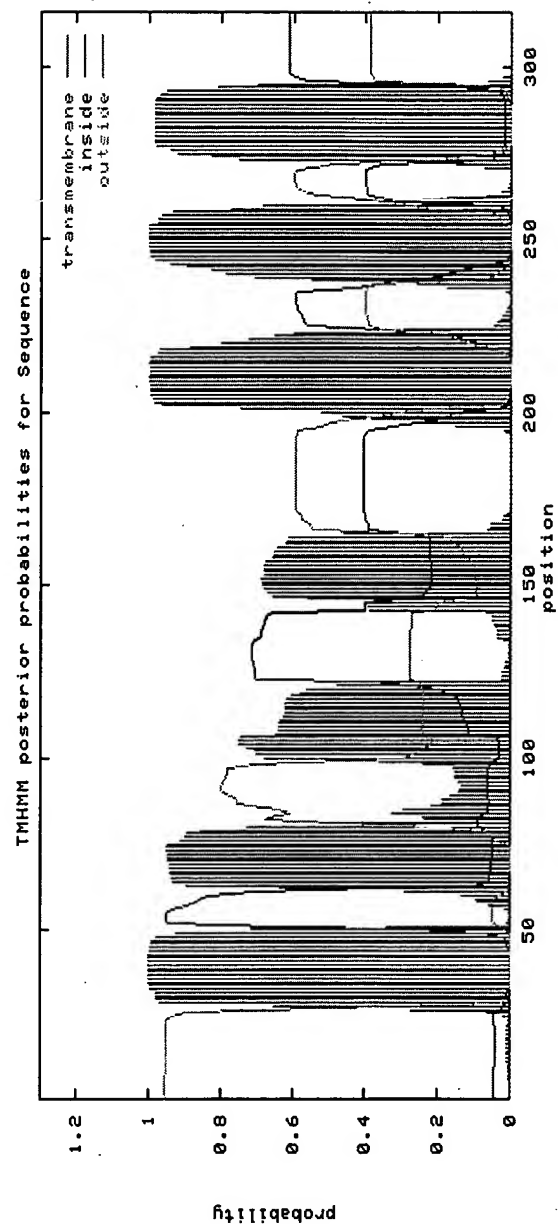


Figure 20. Expression of 101P3A11 in NIH-3T3 Tumors

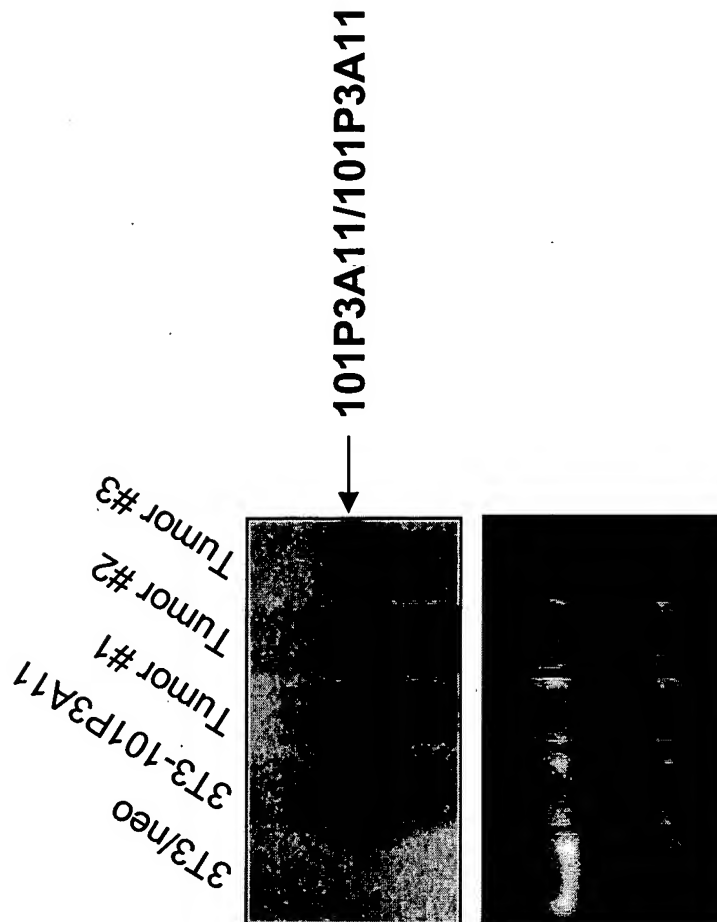
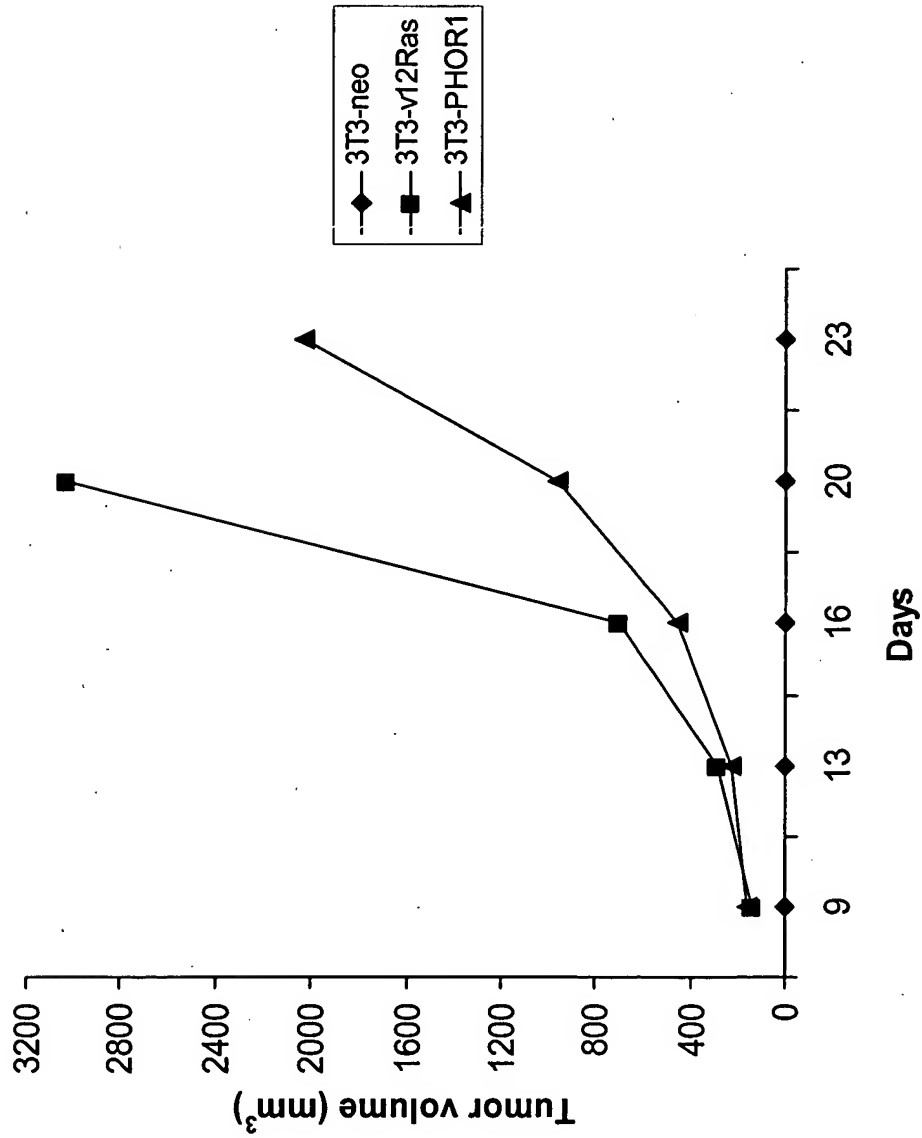
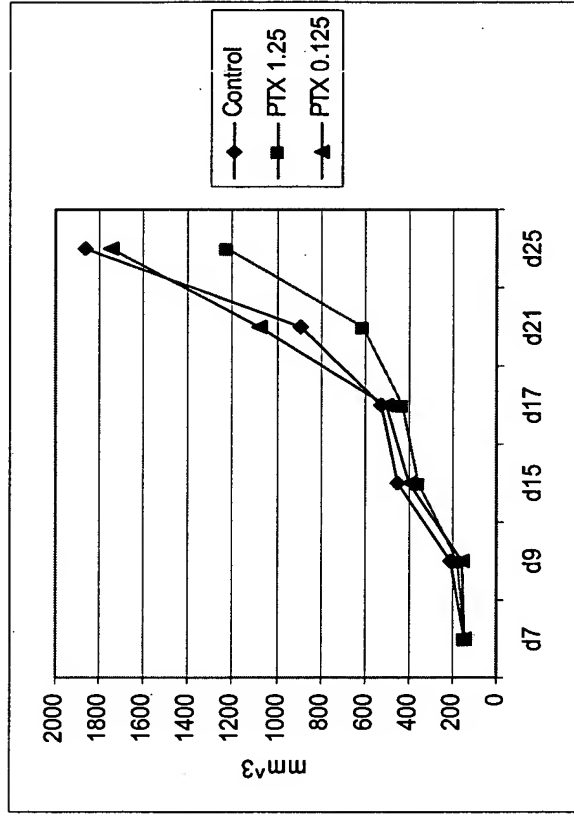


Figure 21: 101P3A11 Induces Tumor Formation of 3T3 Cells



•Injection of 10⁶ 3T3-neo, 3T3-Ras or 3T3-101P3A11 cells subcutaneously into SCID mice revealed that 6/6 3T3-Ras-injected mice formed tumors, 6/6 3T3-101P3A11-injected mice formed tumors, and 0/6 3T3-neo-injected mice formed tumors.

Figure 22: PTX Reduces the *in vivo* Growth of 3T3-101P3A11 Tumors



- Pertussis toxin inhibits the sub-cutaneous growth of 3T3-101P3A11 tumors in SCID mice.
- The inhibitory activity of pertussis toxin occurs in a dose dependent manner.

Figure 23: Alignment of 101P3A11-PHOR-1 with the rat GPCR RA1C (gi|3420759).

Identities = 179/299 (59%), Positives = 231/299 (76%), Gaps = 1/299 (0%)

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PHOR: 14  FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
          F+LIG+PGLEEA FW  FPL S+Y +A+ GN  +++IVRTE SLH PMY+FLCML+ ID+
RA1C: 11  FMLIGIPGLEEAHFWFGFPLLSMYAVALFGNCIVVFIVRTERS LHAPMYLFLCMLAAIDL 70

PHOR: 74  LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR 133
          +STS+MPK+LA+FWF+S  I FDACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR
RA1C: 71  ALSTSTMPKILALFWFDSREITFDACLAQMFFIHALSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDD 193
          HA VL      +IG+ A+VRG+      PLP+ IK+L FC SN+LSHSYC+HQDVMKLA  D
RA1C: 131 HAAVLNNTVTQIGMVALVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLAYTD 190

PHOR: 194 IRVNVVYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCAVFIF 252
          NVVYGL  I+  +G+D +  IS SY LI++ VL L ++  +AKAFGTCVSH+  V  F
RA1C: 191 TLPNVVYGLTAILLVMGVDVMFISLSYFLIIRAVLQLPSKSERAKAFGTCVSHIGVVLA 250

PHOR: 253 YVPFGLSMVHRFSKRRDSPLPVILANIYLLVPPVLNPIVYGVTKEIRQRILRLFHVA 311
          YVP IGLS+VHRF      D  + V++ ++YLL+PPV+NPI+YG KTK+IR R+L +F ++
RA1C: 251 YVPLIGLSVVHRFGNSLDPIVHVLMGDVYLLPPVINPIYGAKTKQIRTRVLAMFKIS 309

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Figure 24: Alignment of 101P3A11-PHOR-1 with the human prostate specific GPCR. (gi|13540539)

Identities = 179/299 (59%), Positives = 233/299 (77%), Gaps = 1/299 (0%)

PHOR: 14 FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73
F+LIG+PGLE+A FW+ FPL S+Y++A+ GN +++IVRTE SLH PMY+FLCML+ ID+
GPCR: 11 FVLIGIPGLEKAHFWVGFPLLSMYVVMFGNCIVVFIVRTERS LHAPMYLFLCMLAAIDL 70

PHOR: 74 LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR 133
+STS+MPK+LA+FWF+S I F+ACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR
GPCR: 71 ALSTSTMPKILALFWFDSREISFEACLTQMFFIHALSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDD 193
HA VL +IG+ AVVRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D
GPCR: 131 HAAVLNNTVTAQIGIVAVVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLAYAD 190

PHOR: 194 IRVNVVYGLVIVIIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCAVFIF 252
NVVYGL I+ +G+D + IS SY LI++TVL L ++ +AKAFGTCVSH+ V F
GPCR: 191 TLPNVVYGLTAILVMGVDVMFISLSYFLIIRTVLQLPSKSERAKAFGTCVSHIGVVLA 250

PHOR: 253 YVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLNPIVYGVTKEIRQRILRLFHVA 311
YVP IGLS+VHRF + V++ +IYLL+PPV+NPI+YG KTK+IR R+L +F ++
GPCR: 251 YVPLIGLSVVHRFGNSLHPIVRVVMGDIYLLLPVINPIIYGAKTKQIRTRVLAMFKIS 309

10001459 = 100101

Figure 25: Alignment with human olfactory receptor 5II12 (gi|14423836)

Identities = 163/304 (53%), Positives = 214/304 (69%), Gaps = 1/304 (0%)

PHOR: 7 NESSATYFILIGLPGLEEAFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLC 66
N + +F+L G+PGLE + WL+ PLC +Y +A+ GN I+ VR E SLHEPMY FL
HOR5: 5 NVTHPAFFLLTGIPGLESSHWSLGSPLCMYAVALGGNTVILQAVRVEPSLHEPMYYFLS 64

PHOR: 67 MLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYV 126
MLS D+ IS +++P +L F N+ I FDACL+Q+F IH S MES +LLAM+FDYRV
HOR5: 65 MLSFSDVAISMATLPTVLRTFCLNARNITFDACLIQMFLIHFFSMMESGILLAMSFDRYV 124

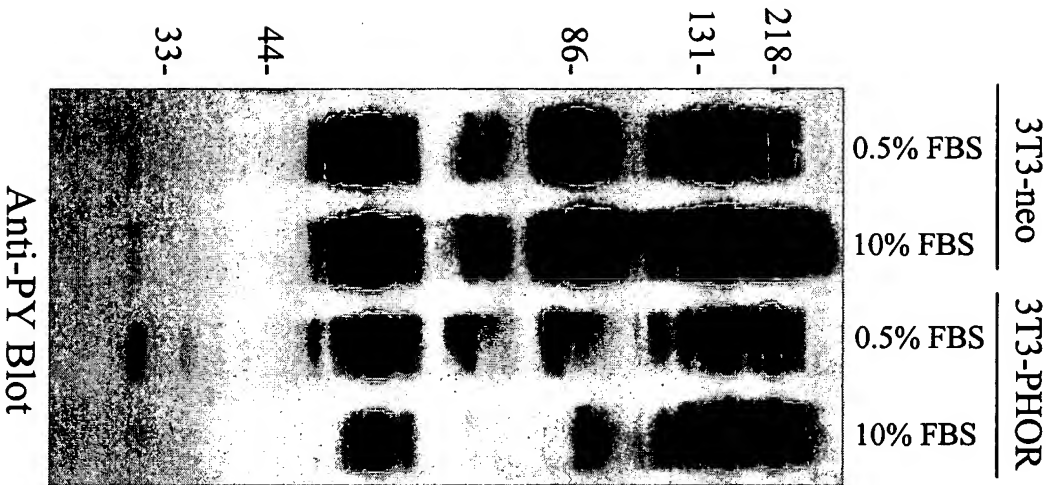
PHOR: 127 AICHPLRHATVLTTLPRVTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDV 186
AIC PLR+ATVLT + +G+ A R + PLP IK+LP CRSN+LSHSYCLH D+
HOR5: 125 AICDPLRYATVLTTEVIAAMGLGAAARSFITLFPPLFLIKRLPICRSNVLSHSYCLHPDM 184

PHOR: 187 MKLACDDIRVNVVYGLIIVISAIGLDSLISFSYLLILKTVLGL-TREAQAKAFGTCVSH 245
M+LAC DI +N +YGL V++S . G+D I SY+LIL++V+ +RE + KA TCVSH
HORS: 185 MRLACADISINSIVGLFVLVSTFGMDLFFIFLSVVLILPSVMATASPEERLKALNTCVSH 244

PHOR: 246 VCAVFIFYVPFGLSMVHRFSKRDSPLPVILANIYLLVPPVLNPVYGVKTKAIRQRIIL 305
+ AV FYVP IG+S VHRF K + V+++N+YL VPPVLNP++Y KTKAIR+ I
HOR5: 245 ILAVLAFYVPMIGVSTVHRFGKHVPCYIHVLSMNLYLFVPPVLNPLIYSAKTKAIRRAIF 304

PHOR: 306 RLFH 309
R+FH
HOR5: 305 RMFH 308

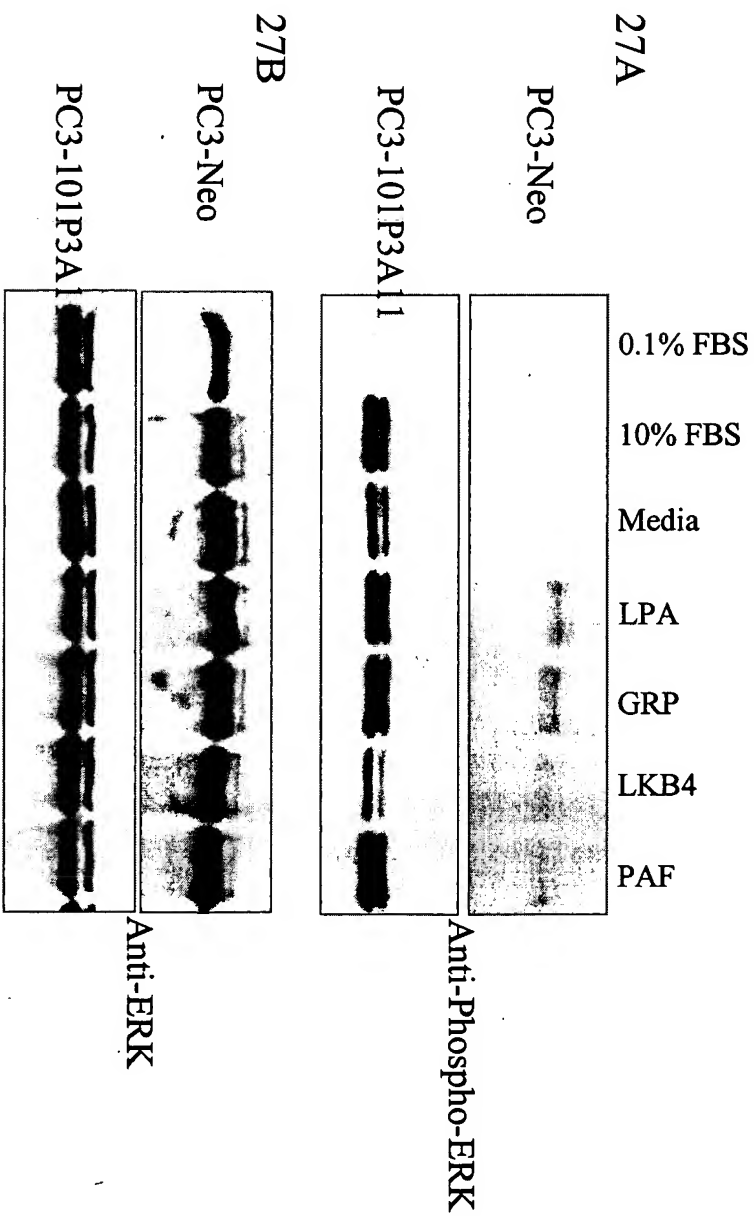
Figure 26: 101P3A11 Modulated Tyrosine Phosphorylation in NIH-3T3 Cells



- 101P3A11 mediated the de-phosphorylation of proteins at 200, 120-140, 85-90 and 55 kDa
- 101P3A11 induced the phsophorylation of proteins at 80 and 29 kDa in NIH-3T3 cells.

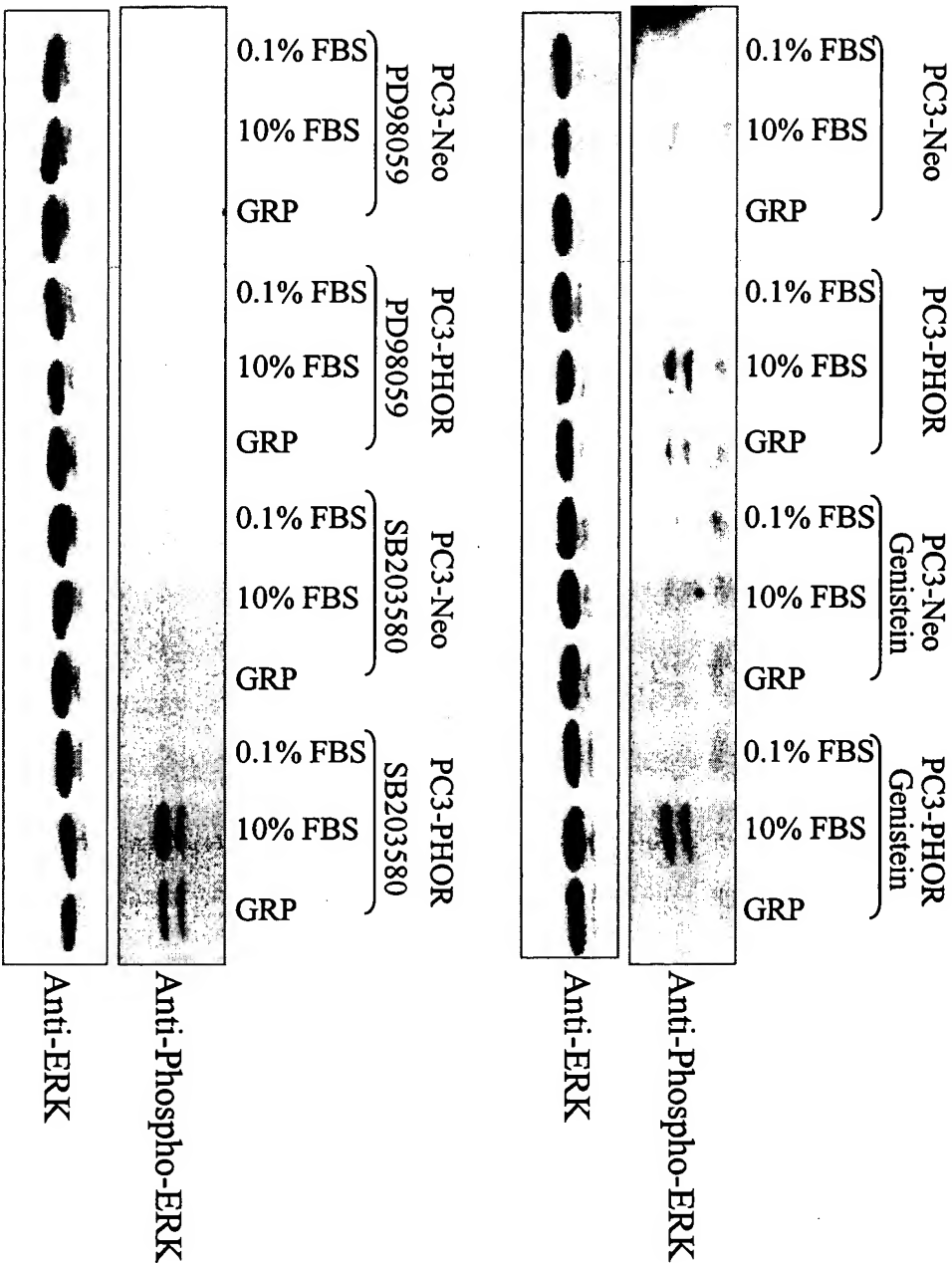
FIGURE 27A-27B

Figures 27A-27B: ERK Phosphorylation by PCR Ligands in 101P3A11
 Expressing Cells



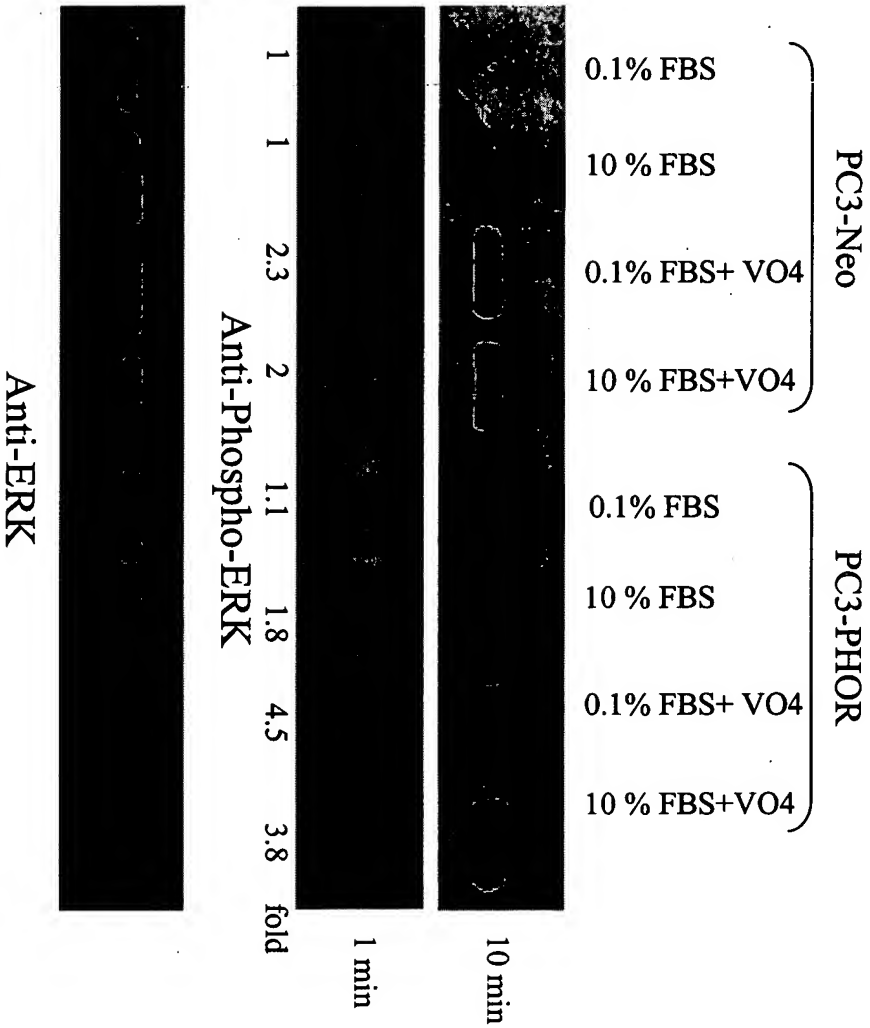
•FBS, lipophosphatidic acid, gastrin releasing peptide, leukotriene and platelet activating factor induced the phosphorylation of ERK in 101P3A11 expressing cells.

Figure 28: Inhibition of 101P3A11-Mediated ERK Activation by PD98059



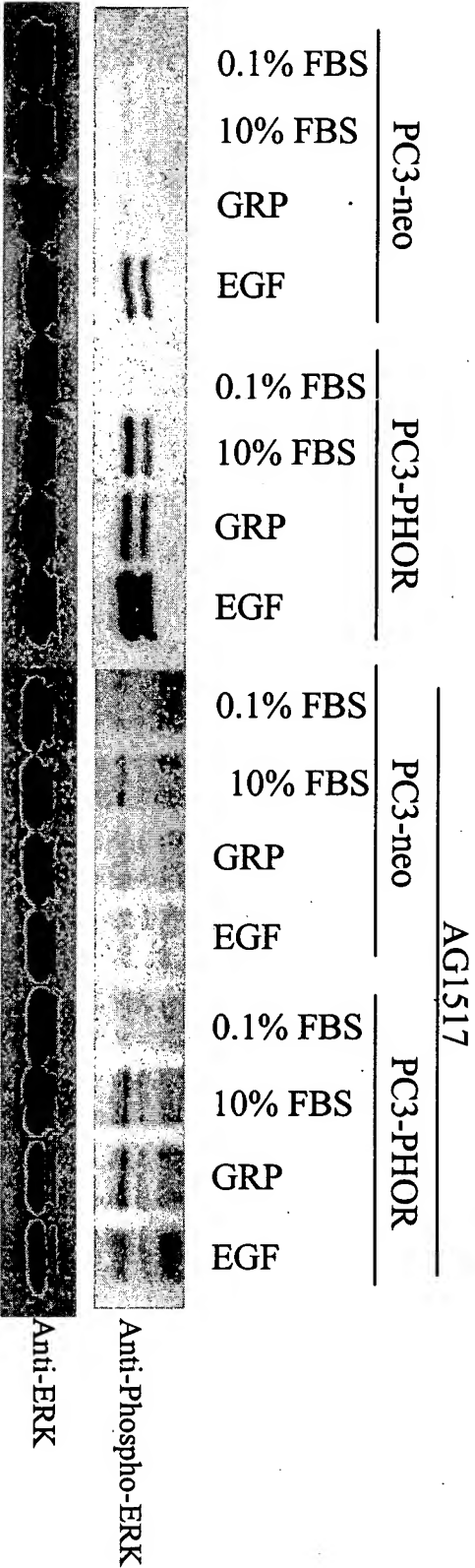
•ERK phosphorylation was inhibited by a MEK specific(PD98059) but not a p38 specific (SB203580) inhibitor in PC3-101P3A11 cells.

Figure 29: Enhanced ERK Phosphorylation in Sodium Orthovanadate Treated
PC3-101P3A11 Cells



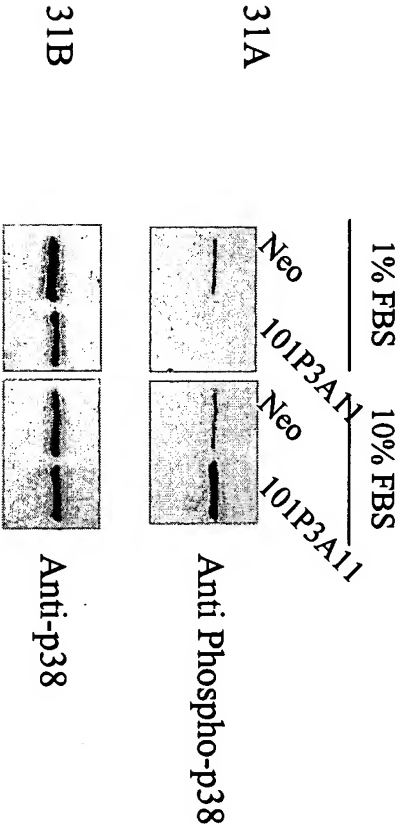
•Sodium orthovanadate induced increased ERK phosphorylation in PC3-101P3A11 cells relative to PC3-neo cells.

Figure 30: Inhibition of 101P3A11-Mediated ERK Phosphorylation
by AG1517



- The EGFR inhibitor, AG1517, inhibits EGF-mediated ERK phosphorylation in control and 101P3A11 expressing PC3 cells.
- AG1517 partially inhibits 101P3A11 mediated ERK phosphorylation in PC3 cells.

Figure 31A-31B: Activation of p38 in PC3-101P3A11 Cells



•Expression of 101P3A11 mediates p38 phosphorylation in cells treated with 10% FBS.

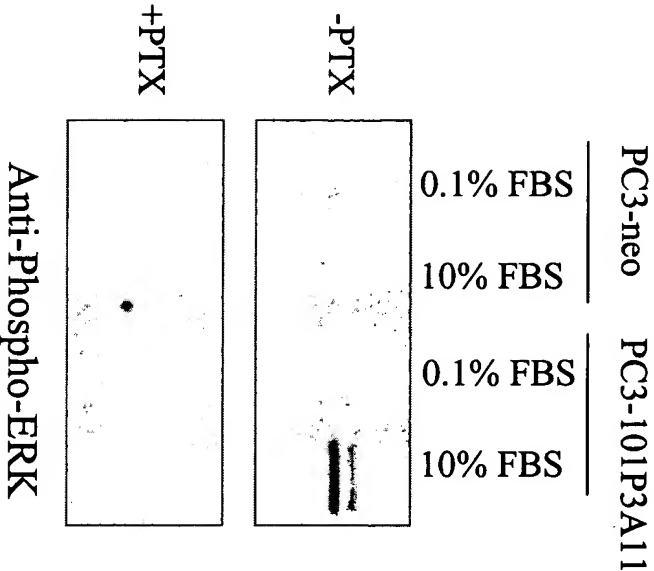
Figure 32: 101P3A11 Induced Accumulation of cAMP in PC3 Cells

Fold change in [cAMP]			
		PC3-Neo	PC3-PHOR
0.1%FBS	-PTX	1	4.302
	+PTX	1.403	2.577
10%FBS	-PTX	2.738	6.978
	+PTX	2.163	2.752

Fold Change in cAMP accumulation was calculated
relative to PC3-neo cells grown in 0.1%FBS

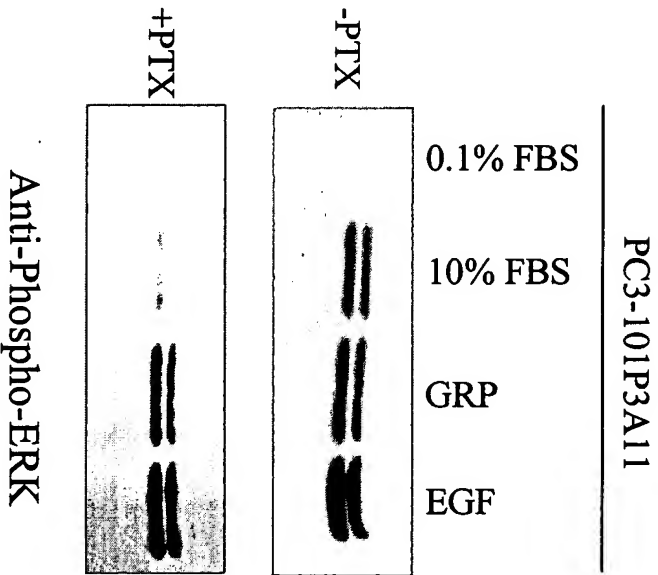
- Expression of 101P3A11 increased the accumulation of cAMP in cells treated with 0.1% and 10% FBS.
- FBS-induced cAMP accumulation in 101P3A11 cells was inhibited by pertussis toxin.

Figure 33: Pertussis Toxin Inhibits 101P3A11 Mediated ERK
Phosphorylation



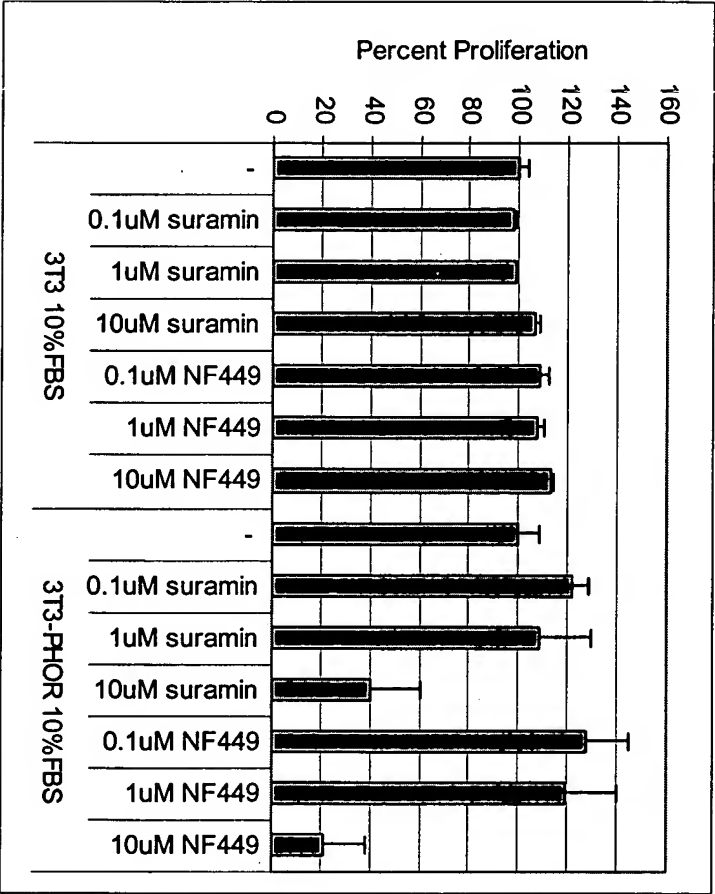
•Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11
expressing cells.

Figure 34: Pertussis Toxin Inhibited ERK Phosphorylation in PC3-101P3A11 Cells



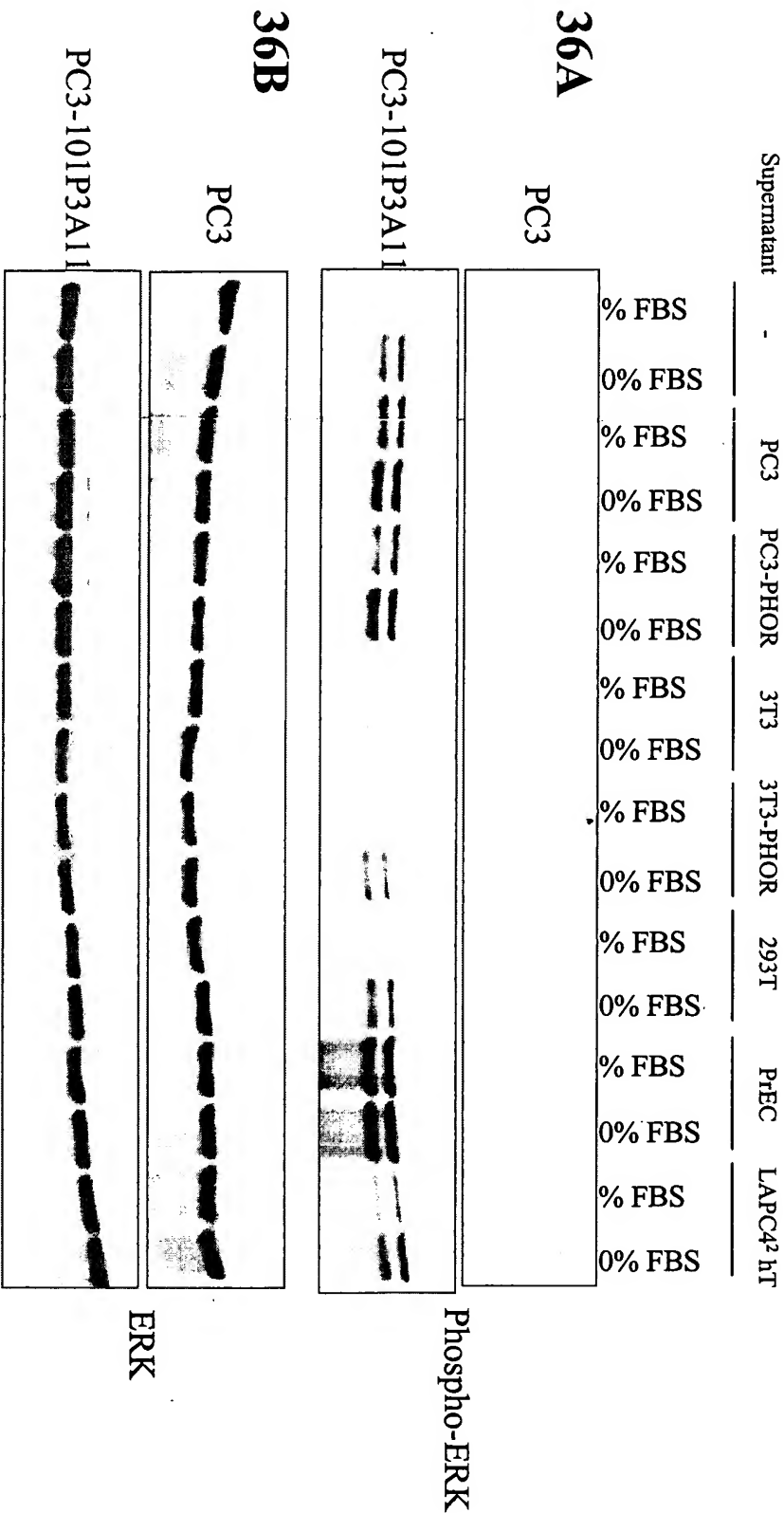
- Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.
- The inhibitory activity of pertussis toxin on ERK phosphorylation was more dramatic in FBS-treated than EGF or GRP-treated PC3-101P3A11 cells.

Figure 35: Inhibition of 101P3A11 Mediated Signaling by Suranim



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence of G protein inhibitors suranim and NF449. Proliferation was analyzed by Alamar blue after 72 hours.
- Suranim and NF449 inhibited the proliferation of 101P3A11 expressing but not control cells.

Figures 36A-36B: 101P3A11 Mediated ERK Phosphorylation By
 Conditioned Media

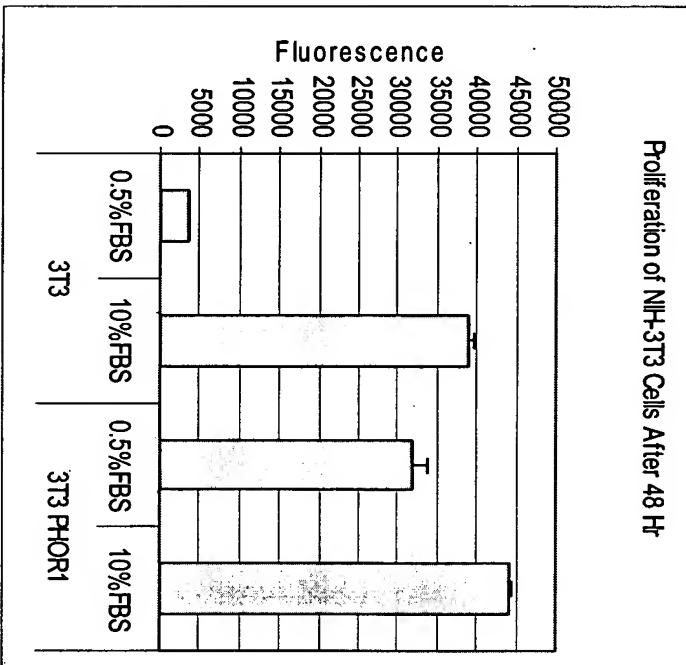


•Supernatants from PC3, PC3-101P3A11, PrEC and LAPC4² cells induce ERK phosphorylation in PC3 101P3A11 but not PC3 cells.

•Supernatants from 3T3 and 293T cells had little specific effect on ERK phosphorylation.

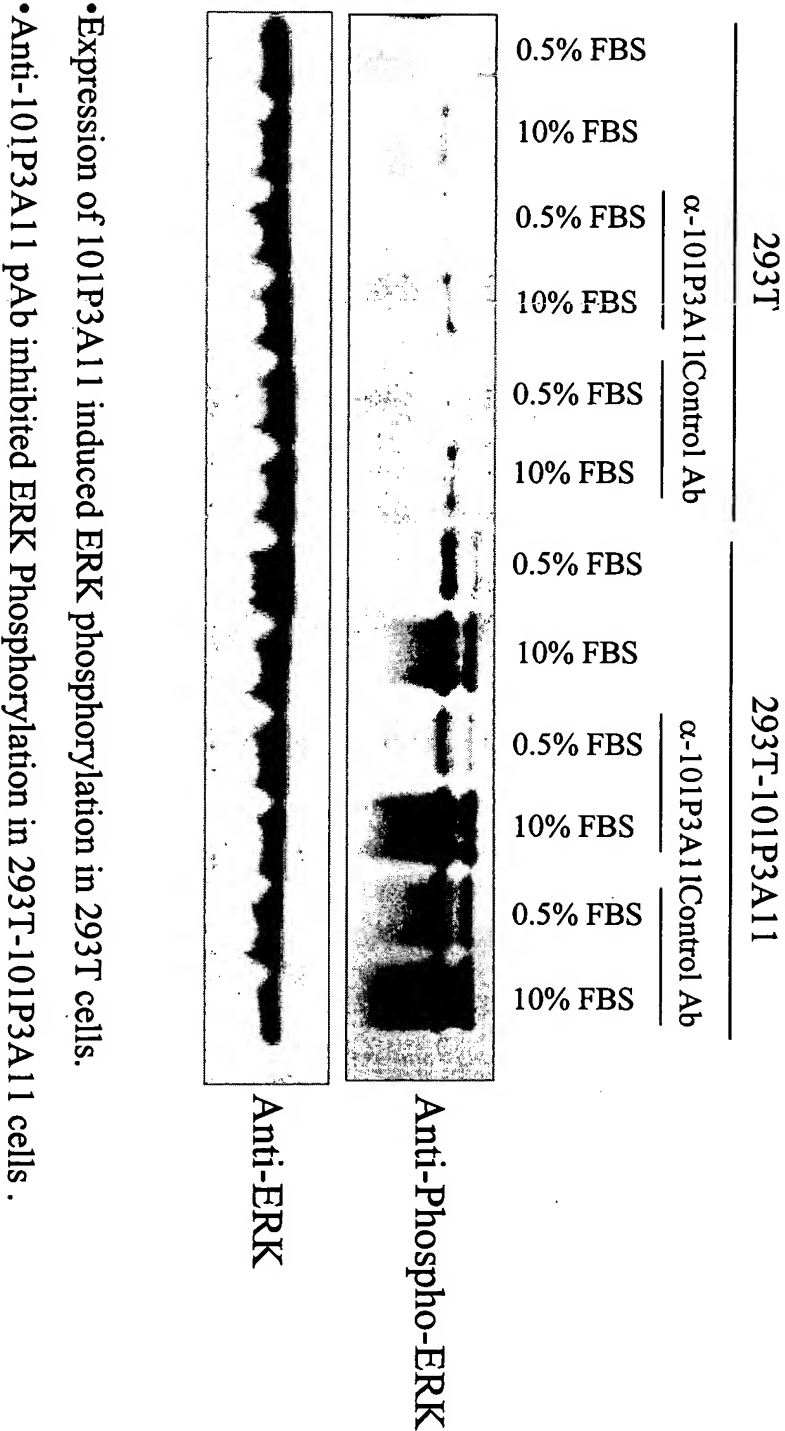
FIGURE 37

Figure 37: 101P3A11 Enhances The Proliferation of 3T3 Cells



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence 0.5 or 10% FBS. Proliferation was analyzed by Alamar blue after 48 hours.
- Expression of 101P3A11 induced a 6 fold increase in the proliferation of 3T3 cells grown in 0.5% FBS.

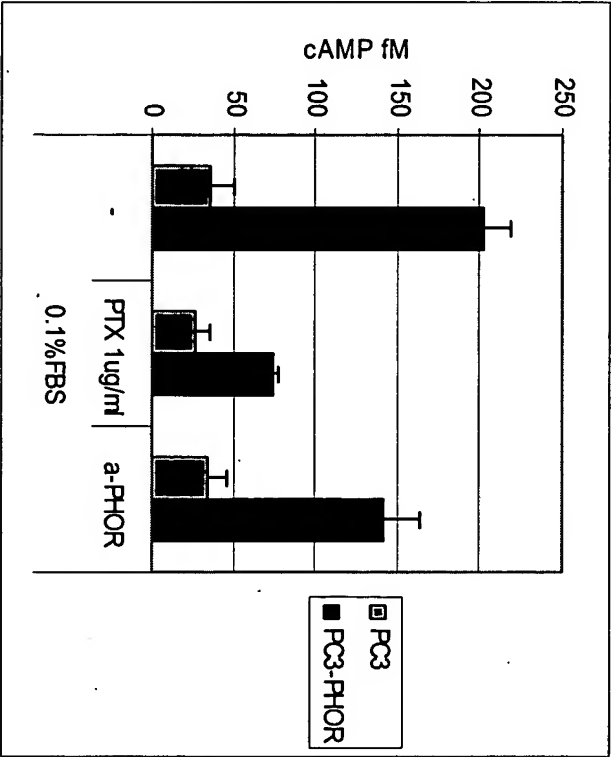
Figure 38: Inhibition of 101P3A11 Mediated ERK Phosphorylation by
101P3A11 Specific Antibodies



- Expression of 101P3A11 induced ERK phosphorylation in 293T cells.
- Anti-101P3A11 pAb inhibited ERK Phosphorylation in 293T-101P3A11 cells .

Figure 39: Anti-101P3A11 Ab Mediated cAMP Accumulation in PC3-101P3A11 Cells

Fold Increase in cAMP			
Treatment	PC3	PC3-PHOR	
0.1% FBS	1 ± 0.42	5.73 ± 0.47	
PTX 1ug/ml	0.74 ± 0.28	2.12 ± 0.09	
anti-PHOR	0.97 ± 0.35	4.01 ± 0.64	



- Control PC3 cells and cells expressing 101P3A11 were treated with anti-101P3A11 pAb for 2 min and evaluated for intracellular cAMP content.
- The assay was performed in duplicate.

Figure 40A-40F

Fig. 40A. Prostate Cancer, 400X

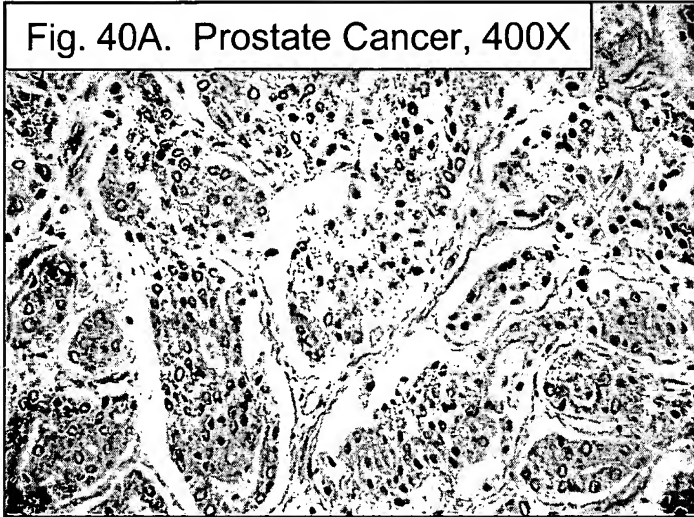


Fig. 40D. LNCaP, 400X

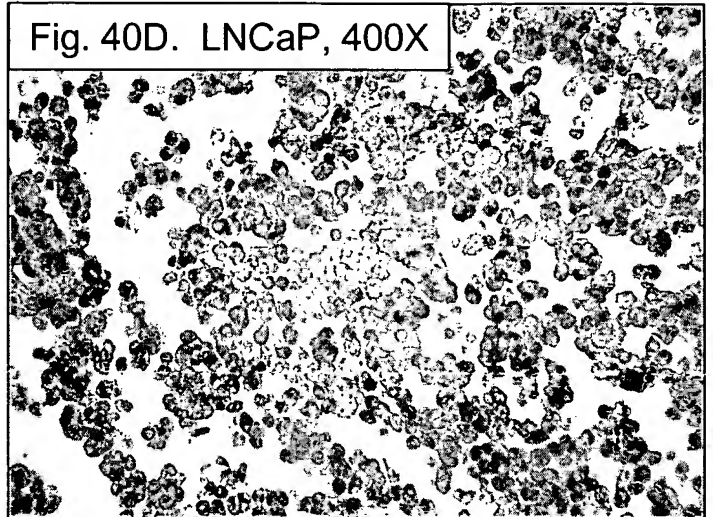


Fig. 40B. Prostate Cancer, 400X

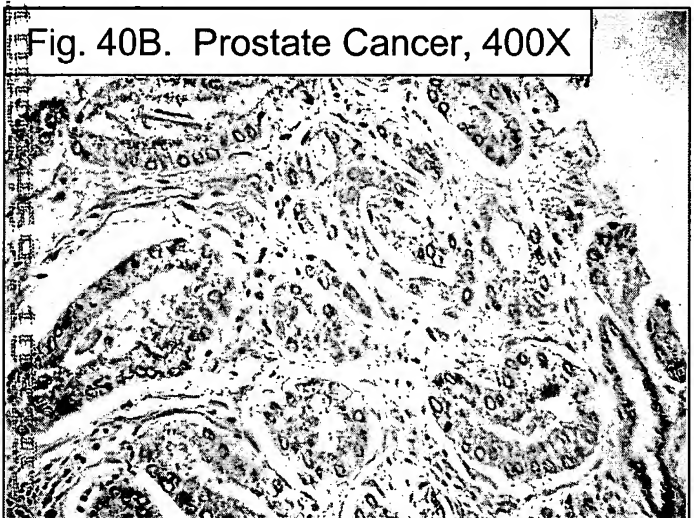


Fig. 40E. Prostate, 400X

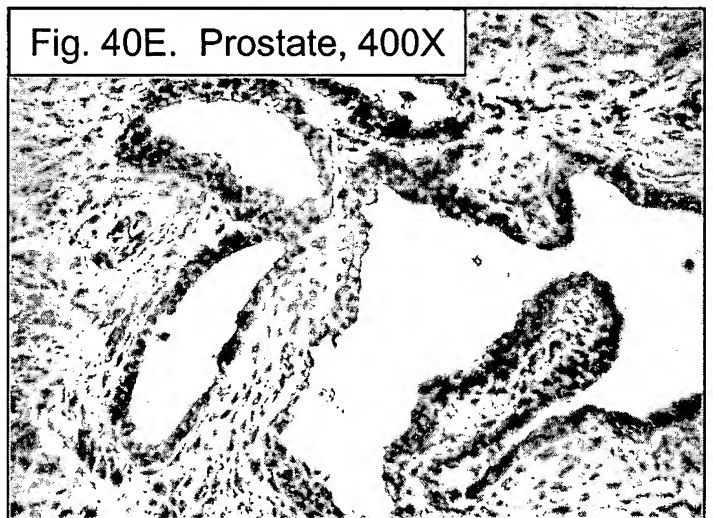


Fig. 40C. Prostate Cancer, 2000X

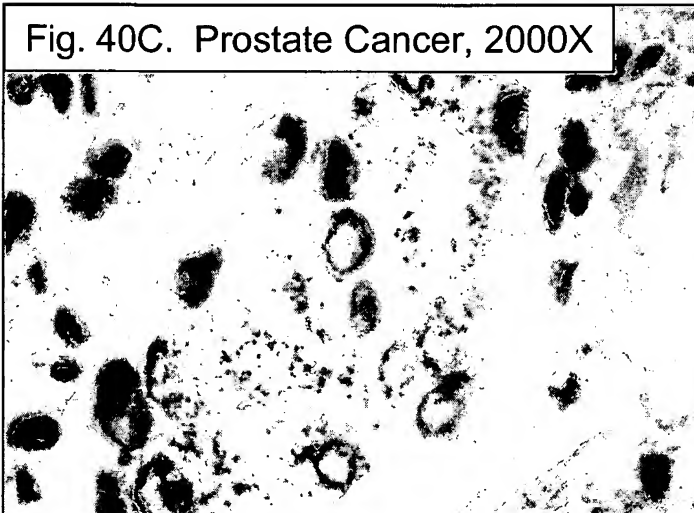


Fig. 40F. Prostate, 2000X

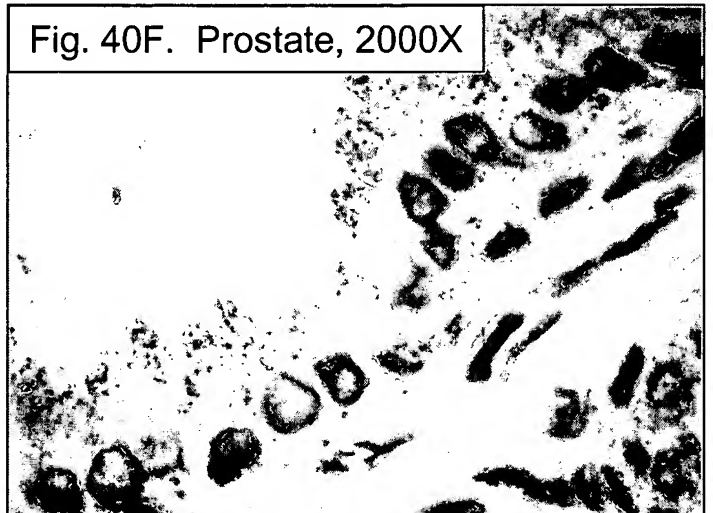


Fig.41A Prostate Cancer, 800X

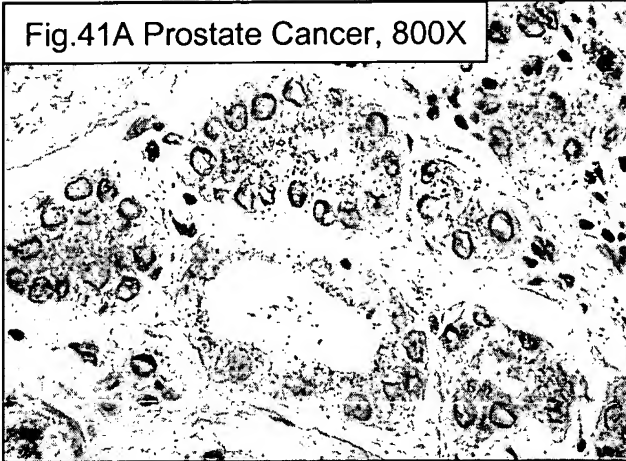


Fig.41B Bladder Cancer, 800X

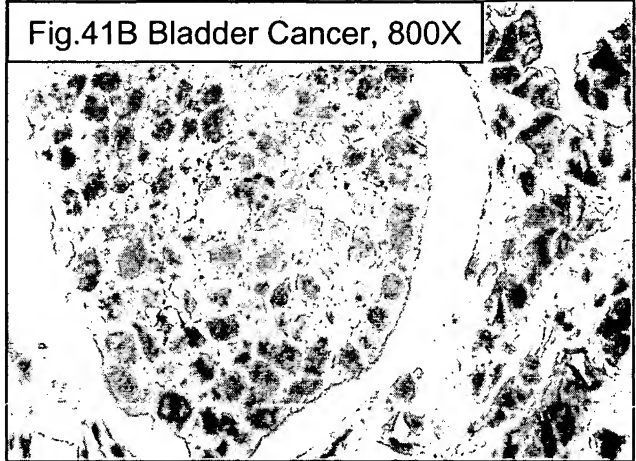


Fig.41C Kidney Cancer, 800X

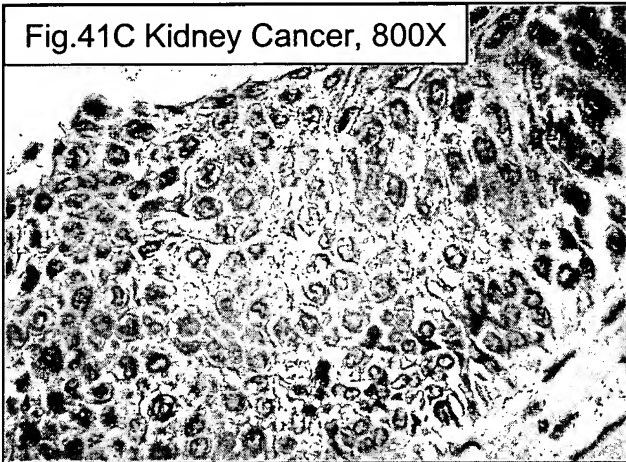


Fig.41D Colon Cancer, 800X

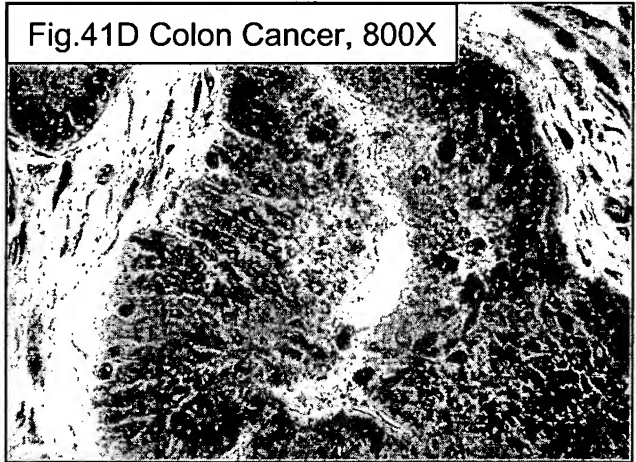


Fig.41E Lung Cancer, 800X

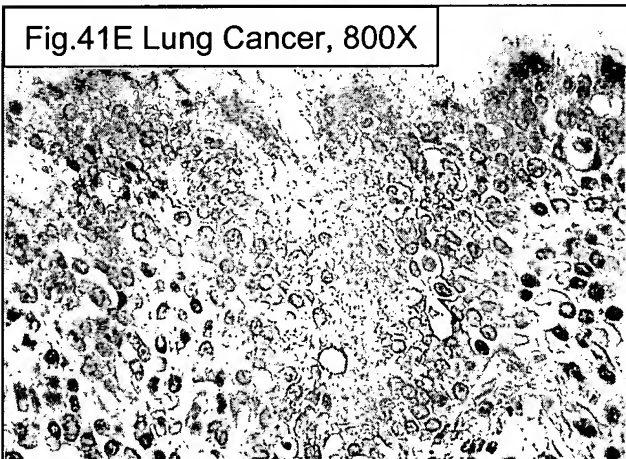


Fig.41F Breast Cancer, 800X

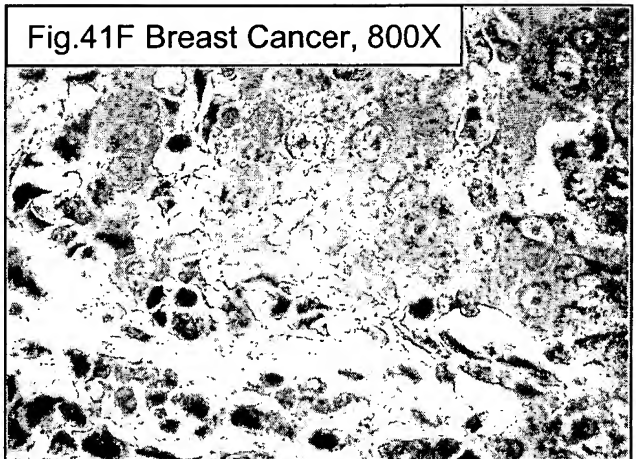
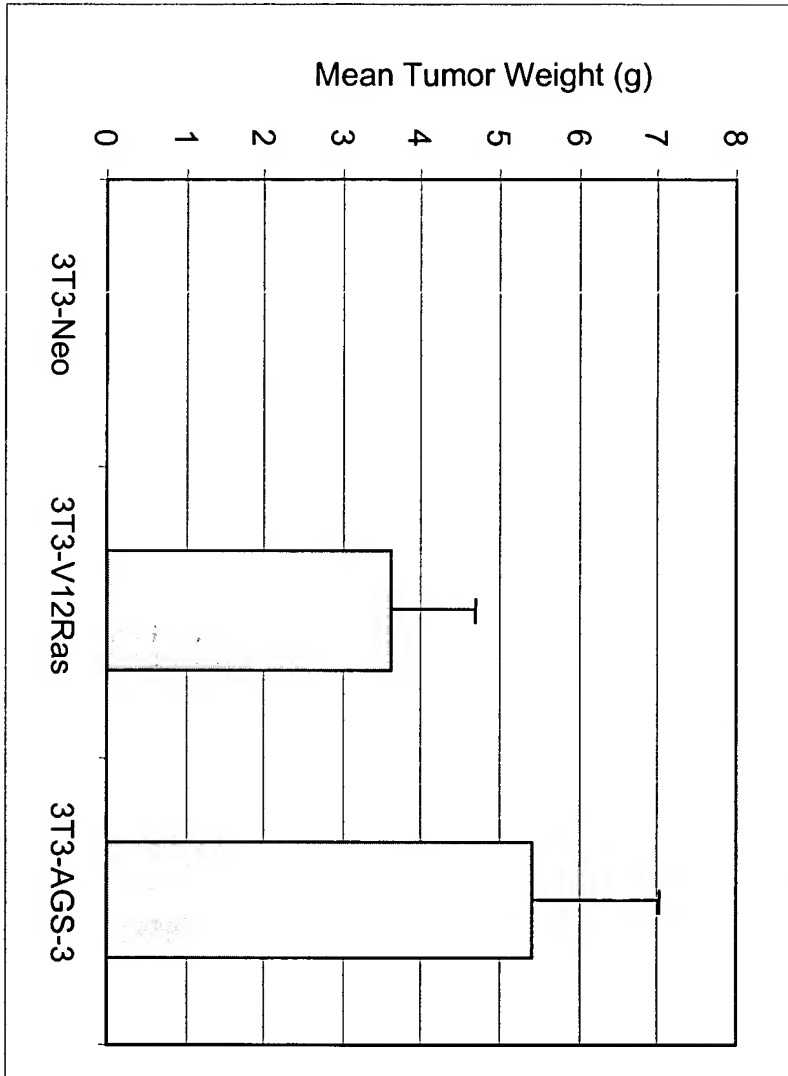
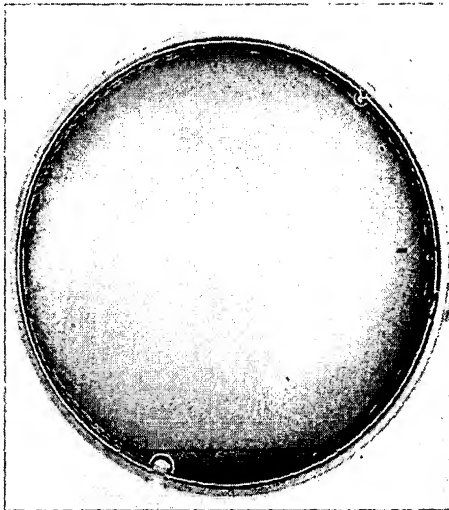


Figure 42

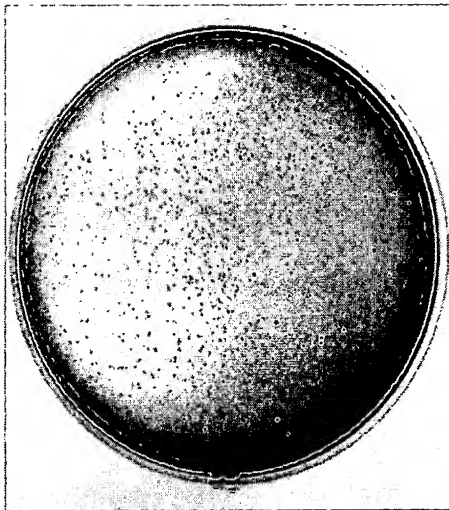


101P3A41-00001

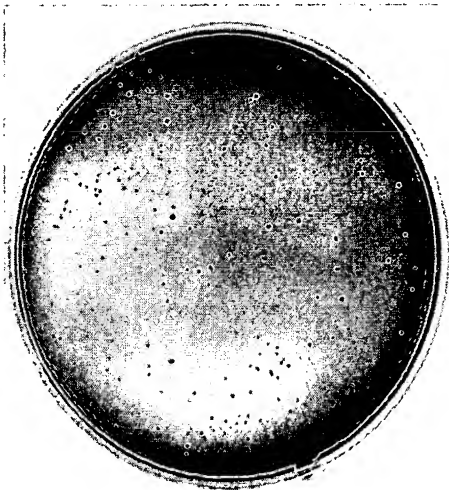
Figure 43



Neo



101P3A11



Ras

Cell Line	Colony number
	Average
3T3-neo	0.5
3T3-101P3A11	686
3T3-Ras	249